THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME VII MARCH, 1931 NUMBER 2

PRIMARY LIPOSARCOMA OF BONE *

FRED W. STEWART, M.D.

(From the Pathology Laboratory, Memorial Hospital, New York City)

Out of the heterogeneous group of primary bone tumors have gradually emerged certain more or less defined clinical pathological entities — the benign giant cell tumors, the osteogenic sarcomas of varying structure, origin, course and prognosis, the endothelial myeloma of Ewing, primary angioendothelioma, various types of myeloma, erythroblastoma and possibly primary lymphosarcoma of bone. The only constituent of bone or bone marrow unrepresented in the tumor field seems to be the fat tissue which makes up such a large portion of quiescent marrow.†

In the extensive collection of bone tumors at the Memorial Hospital, opportunity has been afforded for the study of many varieties of bone tumors. From time to time the laboratory has been puzzled in properly classifying certain types of perithelial, alveolar or diffuse bone tumors. The tendency for a time was to regard such tumors as metastatic, and various organs, notably adrenal, kidney and thyroid, have been incriminated as the primary focus of disease. Nevertheless both clinical and autopsy evidence indicate that there exists a certain small group of pseudo-epithelial, spindle or polyhedral cell, diffuse or alveolar tumors of bone which are not metastatic, and there are reasons to believe that such tumors take their origin in the fat tissue of the marrow and are therefore liposarcomas. In one tumor which we have regarded as a liposarcoma the cells are diffusely

* Received for publication January 13, 1931.

† Nienhuis, J. H. (*Ztschr. f. Krebsforsch.*, 1925, 22, 434), describes bone involvement in liposarcoma, but in reviewing his case it is apparent that he regards the bone tumors as metastatic from a primary, or possibly multiple primary soft part tumor of fat tissue.

arranged and clear, with small peripheral nuclei like fat cells. In another they are both diffuse and alveolar, very large, with central or peripheral nuclei and show a cytoplasm filled with small xanthoma-like droplets. There are many tumor giant cells. In neither of these cases was the material available for fat stains at the time the diagnosis of liposarcoma was suspected. In the third case, however, the biopsy was so suggestive that when the case finally came to autopsy, tissues were stained with Sudan III and the alveolar cells were found to be loaded with finer or coarser fat droplets. In ordinary osteogenic sarcoma the cells contain practically no fat. The author is very hesitant in advancing the notion that these tumors are liposarcomas and has withheld publication of these cases in the hope of gathering more conclusive material at some subsequent date. However, examples of this disease are rare and it was at last decided that these cases should be published to enable other pathologists to look for similar tumors. Brief summaries of the three cases follow.

Case Reports

Case 1. G. P., male, aged 33 years, entered the Hospital July 26, 1926. His history prior to the onset of the present illness was uneventful. Four years ago (1922) a swelling appeared on the inner aspect of the third finger of the right hand. The finger was amputated at Bellevue Hospital, a diagnosis of sarcoma was made, and the patient remained free from further symptoms until eighteen months later. At that time there was slight swelling of the right hand and forearm which progressed steadily until admission to the Memorial Hospital. At the time of admission the forearm was diffusely swollen and radiographs revealed complete, or nearly complete destruction of the radius, slight destruction of the ulna and pathological fracture. Radiographs of the chest revealed no metastases. The arm was amputated.

Gross Description of Tumor: Arm amputated through lower humerus. The radius was replaced by a huge tumor mass (Fig. 1) fusiform in shape, 22 cm. long and 13 cm. in diameter, displacing the muscles but encapsulated by a distended periosteum. Of the bone, but the upper 2 cm. and the lower 1 cm. remained. The tumor seemed to have developed in the radius and to have replaced almost the entire bone. It was cystic in the center, 70 cc. of straw-colored fluid having been aspirated. About this central cavity the tumor mass was necrotic and this necrosis extended nearly out to the capsule. Especially along the lower end there were some soft, cellular, apparently growing areas. The main artery crossed over the tumor

with no definite entrance into it. The metacarpal bone of the amputated phalanx was normal.

Microscopic Examination: The sections show a very peculiar tumor made up in part of small hyperchromatic, spindle and polyhedral cells. Much of the material was unsuited for study on account of necrosis. However, in some regions (Fig. 3) the tumor is comprised of large clear cells with small, generally peripheral and at times flattened, hyperchromatic nuclei resembling fat tissue. The diagnosis of liposarcoma is based partly on these areas and partly on the clinical course.

Comment: The tumor is certainly not the usual bone tumor. It began apparently in a phalanx where we do not see osteogenic sarcoma. It did not recur locally but developed either by venous extension or entirely *de novo* in another bone of the same extremity, grew to enormous size but failed to metastasize. It did not behave like any osteogenic sarcoma or endothelial myeloma we have ever seen. It was not metastatic since it is quite inconceivable that the tumor could have grown to such bulk in two different bones and over so long a period, with the primary tumor, if such there were, remaining symptomless. Furthermore, the patient is still well. In other words we have a bone tumor arising in a peculiar location, showing a picture histologically suggestive of a fat tumor, no evidence of any other primary lesion and a very unusual clinical course. We believe the diagnosis of liposarcoma is justified in this case.

CASE 2. The second case was a male, aged 28 years. He entered the Memorial Hospital August 29, 1924. His illness was of approximately one year's duration, with the initial symptom pain in the calf of the left leg. A physician, apparently noting some swelling, had attempted to aspirate the lesion. Nothing was obtained and the pain increased. The patient entered a hospital in Sayville, Long Island, where deep in the left calf a smooth mass some 5 cm. in diameter was noted. The mass was thought to be in the soft tissues. It did not pulsate but yielded a bruit on auscultation. A popliteal aneurysm was considered in the differential diagnosis. X-rays were reported negative. The patient was operated upon and the surgeon removed what he considered to be the whole tumor. He reported it encapsulated but adherent over the posterior surface of the head of the fibula, that is, closely applied to bone but not arising from bone. A pathologist rendered the report "alveolar sarcoma." By July of 1924 there was a definite recurrence. On admission to the Memorial Hospital the head of the fibula was the site of a globular, pulsating, tense tumor mass 5 cm. in diameter. Over the anterior surface of the left tibia at the junction of its middle and lower thirds was a second tumor producing diffuse swelling, slight edema and very marked tenderness, covering an area 8 by 5 cm. Radiographs of the chest re-

vealed pulmonary metastases. On June 2, 1926, since the chest metastases appeared stationary and since a pathological fracture of the tibia, present on admission, had failed to unite under treatment, an amputation was performed.

Gross Description of Tumors: The tumors were situated as described in the clinical summary. As in the preceding case, they apparently arose in the bone (Fig. 2), distending and pushing aside the soft part structures but remaining completely encapsulated. The upper tumor measured 9 cm. in diameter, the lower 14 cm. in length and 8 cm. in diameter. They were of a peculiar light yellow color, soft, but coherent. The lower femur, os calcis and small bones of the foot were the seat of numerous soft, hemorrhagic lesions grossly resembling angiomas, and the lesion was thought to be an angioendothelioma.

Microscopic Examination: The histology in this case is very bizarre. The tumor is made up in part of large, loosely arranged cells with relatively small, central or peripheral, deeply hyperchromatic nuclei (Fig. 5). In the cytoplasm are numerous xanthoma-like droplets which for the most part do not fill the entire cell. These peculiar cells do not line blood vascular channels. In other areas the tumor is comprised of small, closely packed spindle cells (Fig. 6). Sections from the above described vascular lesions of the marrow of other bones apart from the main tumor reveal small hemorrhagic foci of the fat tissue (Fig. 7). There is polymorphonuclear invasion of the fat tissue, some mucinous degeneration and the appearance of "young" fat cells. The process shows no neoplastic features but nevertheless appears to be rather significant in that this multiplicity of the vascular lesions of the other bones suggests one case of soft part liposarcoma observed during the past year. This tumor had developed in the soft tissues of the lower extremity, and in the amputated specimen in addition to the main tumor which was clearly liposarcomatous, there were peculiar fat changes all along the leg from ankle to knee. The fat lobules were unusually small, the cells were small and there was a diffuse nodular injection although the individual fat nodules showed no neoplastic tendencies.

Comment: In this second bone tumor the diagnosis of liposarcoma is based upon the peculiar gross appearance, alveolar character, xanthoma-like droplets in the individual cells, absence of evidence of origin from endothelium, lack of resemblance to any known variety of primary bone tumor and the clinical course, *i.e.*, the ab-

sence of evidence of any primary epithelial tumor over a period of six and one-half years. The clinical course was peculiar in that the pulmonary metastases remained well under control by radiation up to the time of death, more than five years after admission. Death was apparently due to intracranial metastases.

CASE 3. G. H., male, aged 28 years, entered the Memorial Hospital on April 4, 1930. It was almost impossible on account of the patient's nationality to determine the sequence of events in the course of his illness. A short time prior to his admission at Memorial he had attended another hospital for diagnosis, and on the basis of radiographs his ailment was thought to be due to multiple myeloma involving the right femur, the left parietal bone, right seventh rib and midthoracic vertebrae. To establish the diagnosis an exploration of the femoral tumor which had produced a large soft part swelling was performed. The mass was soft, pinkish and very vascular. A portion of the tumor was excised for diagnosis but no positive opinion could be rendered. Our own diagnosis on the slides from this operation was inconclusive. The possibilities were in order (1) primary alveolar liposarcoma of bone, (2) angioendothelioma, (3) metastatic thyroid or adrenal carcinoma. In this report we called attention to the resemblance between this case and Case 2 of this series. Under X-ray treatment the tumors proved very radiosensitive. Nevertheless the disease was far advanced on admission. Evidence of spinal cord compression was apparent. The patient was incontinent and died eleven days after admission. Complete autopsy was performed, including the neck organs, and no primary tumor could be discovered.

Postmortem Examination: There was a diffuse subcutaneous swelling above and behind the left ear as one frequently sees overlying destructive tumors of the cranial bones. Beneath it an area of bone destruction in the parietotemporal region could be palpated. The tumor was soft, reddish, pale brick-colored, vascular, granular and sharply demarcated from the surrounding bone.

The left lung and pleura were studded with very small metastatic nodules, pinkish and vascular. There was a soft, pinkish mass, ovoid in shape, tense and elastic, measuring about 4 by 4 by 8 cm. overlying the sixth, seventh and eighth ribs adjacent to the vertebral column on the right. On section this mass was soft, brick red in color and pulpy. It extended into the vertebrae and a finger introduced into the tumor could be passed directly down to the spinal cord. Aside from the tumor of the femur no other lesions were found. The latter tumor was situated at the junction of the upper and middle thirds of the femur. No soft part mass was found (the tumor had regressed under radiation). The outer cortex of the bone was roughened but otherwise normal. The tumor occupied the

medullary cavity. On one aspect the cortex was not involved, but on the other the neoplastic tissue eroded bone and extended nearly to the surface. The material again was soft, rather pulpy and brick red in color and was sharply demarcated from the surrounding marrow which was very fatty.

Microscopic Examination: Sections reveal a tumor suggesting an alveolar carcinoma. The alveolar structure is well developed. The cells are irregular in size, rather polyhedral in shape; some are opaque, others coarsely or finely vasculated. This alveolar appearance is maintained in all of the metastases. The stroma is fibrous and in places fairly abundant. Sudan III stains show that the tumor cells are loaded with fat in coarser or finer droplets (Fig. 4). Many of the cells approach the large polyhedral cells observed in the preceding case.

Comment: The diagnosis of liposarcoma in this instance is based on the absence of primary tumor in a thoroughly autopsied case, the abundant fat, and the lack of resemblance to any other known tumor.

Summary and Conclusions

In reporting these three cases of bone tumor as liposarcomas we are fully cognizant of the fact that we are in doubtful territory. In no case has it been possible to trace the actual origin of the tumor to fat cells, although in one instance the inflammatory fat changes in the marrow apart from the tumor were very suggestive. The conclusions drawn rest entirely on circumstantial data: (1) the resemblance of the tumor cells to fat cells, the presence of large fat droplets in one case, the general resemblance to fat cells in another although no specific fat stains are available, and the xanthomatous droplets in the second case; (2) the peculiar clinical course, namely, a suggestion at least of multiplicity of bone lesions in all three instances which recalls the behavior of certain of the liposarcomas we ourselves have observed in the soft parts; (3) lack of evidence of primary epithelial origin, and (4) an appearance inconsistent with primary bone tumors of known types.

That the disease is more or less of a clinical entity may be surmised from the fact that all three of the tumors were either multiple in bone or else showed a pronounced tendency to metastasize to other bones, and that of the two cases treated by irradiation both

proved radiosensitive. The bony tumors regressed very markedly in the one and the pulmonary metastases were long held in check in the other. This radiosensitivity is quite inconsistent with either a primary bone tumor of the osteogenic variety which even remotely approaches our liposarcomas in structure, or with a metastatic adenocarcinoma, but it is not inconsistent with certain liposarcomas we have observed in the soft tissues. It is of further interest from the clinical standpoint that whereas in osteogenic sarcoma cranial metastases are rather uncommon, in two of the three tumors we have regarded as liposarcoma cranial involvement has occurred.

DESCRIPTION OF PLATES

PLATE 18

FIG. 1. Amputated arm, Case 1. Middle finger previously amputated at another hospital. Destructive, semicystic tumor involving nearly the entire radius.

FIG. 2. Tibia, Case 2. Shows the lower tumor only.

1

2

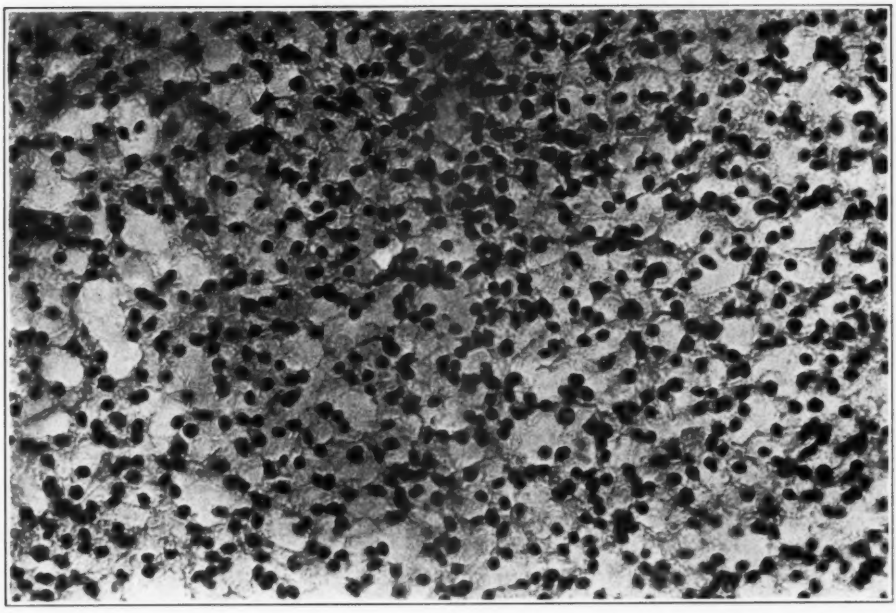
Stewart

Primary Liposarcoma of Bone

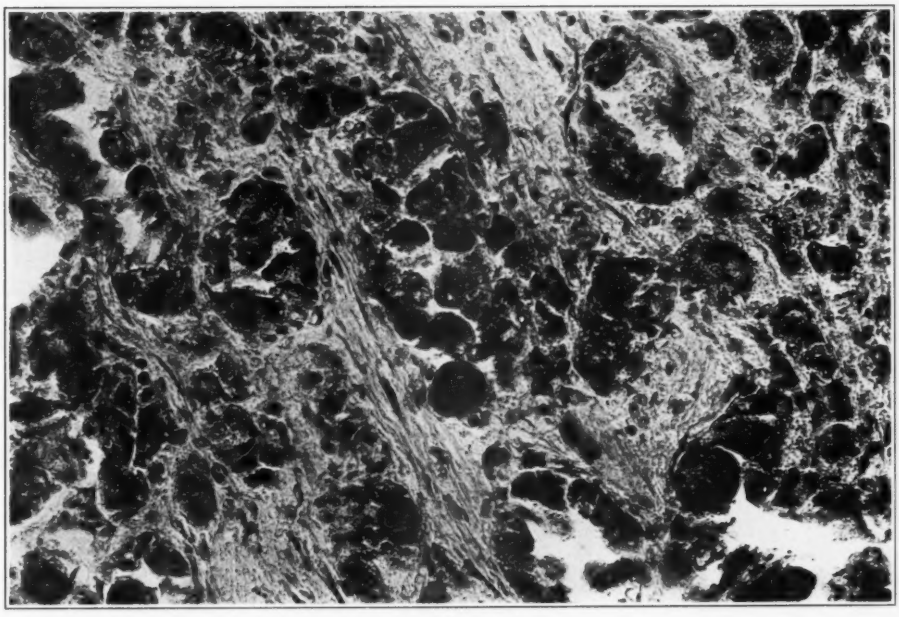
PLATE 19

FIG. 3. Case 1. Diffuse tumor mass in which the cells resemble small fat cells.

FIG. 4. Case 3. Tumor cells in alveolar arrangement. Marked variations in size and shape. Much Sudan III stainable fat.



3



4

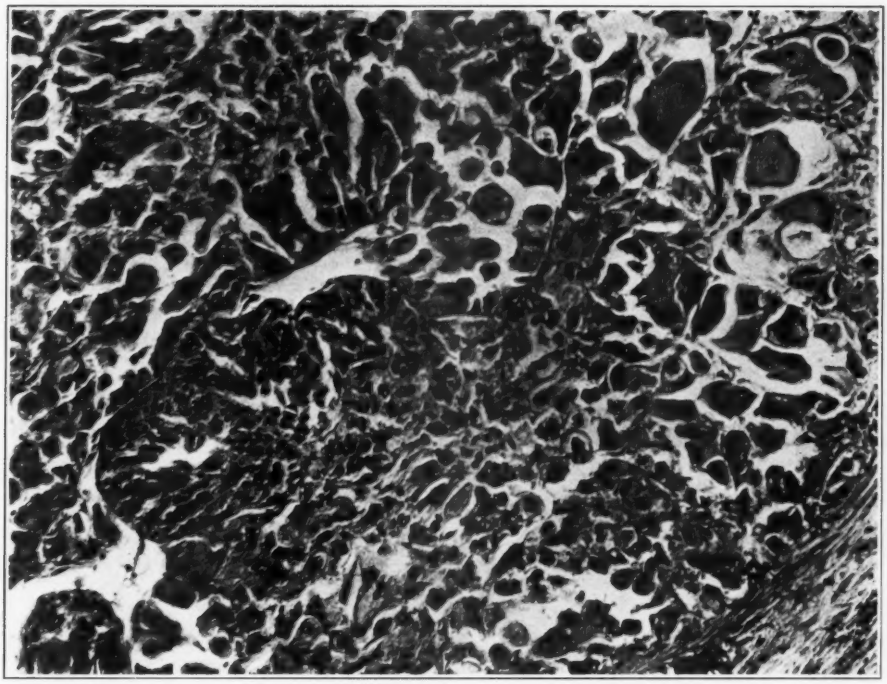
Stewart Primary Liposarcoma of Bone

PLATE 20

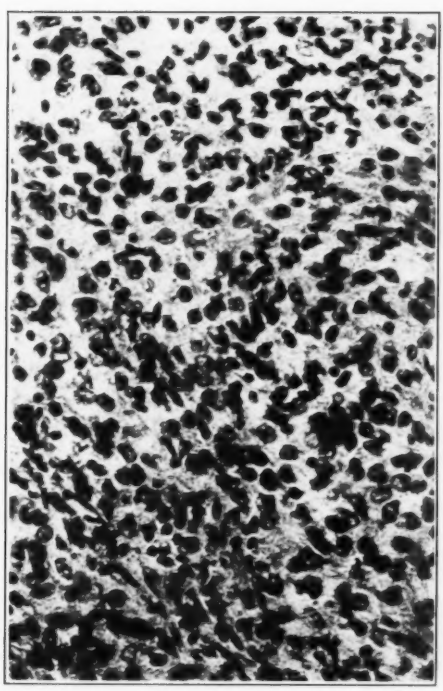
FIG. 5. Case 2. Masses of large polyhedral cells in vaguely alveolar formation. Marked variation in size and shape. Nuclei often peripheral. Numerous xanthoma-like droplets.

FIG. 6. Case 2. Another area showing the tumor comprised of numerous small spindle cells.

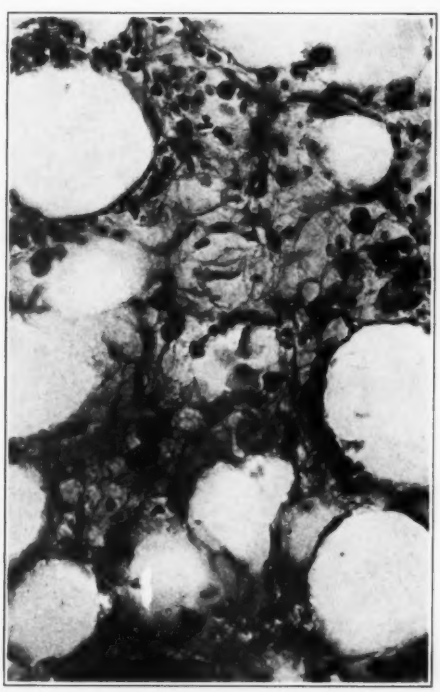
FIG. 7. Case 2. Cells taken from the hemorrhagic areas apart from the tumor. These show the morphology of young fat cells. There is mucinous degeneration of the marrow and invasion of some of the fat cells by polymorphonuclear leukocytes.



5



6



7

Stewart

Primary Liposarcoma of Bone

PATHOLOGICAL STUDY OF A CASE OF ENDEMIC TYPHUS IN VIRGINIA WITH DEMONSTRATION OF RICKETTSIA *

HENRY PINKERTON, M.D., AND KENNETH F. MAXCY, M.D.

(From the Department of Pathology, Harvard Medical School, Boston, Mass., and the Department of Public Health and Hygiene, University of Virginia, Richmond, Va.)

INTRODUCTION

Endemic typhus is definitely established as a disease entity in the United States, and the number of cases occurring in recent years (Maxcy [1]) has been sufficiently large to make the condition of considerable medical interest.

When Brill[2] in 1910 reported 120 cases of typhus in New York, the nature of the condition was so uncertain that he preferred not to give it a definite name. In 1912, Anderson and Goldberger[3] infected monkeys with the virus of this "unknown disease" and showed by cross immunity tests that it was in all probability essentially identical with Mexican typhus, but this evidence was not universally accepted.

In 1923, when Maxcy began his studies of the problem, he was aided greatly by the Weil-Felix reaction, and also by the fact that the experimental pathology of the disease in the guinea pig had been worked out. He was able repeatedly to establish the disease in guinea pigs and demonstrated the complete identity of his strains with Mooser's strain of Mexican typhus, and the essential identity (cross immunity) with two strains of European typhus. The differences between the Old World and New World strains in the guinea pig will be referred to later.

Maxcy has presented strong epidemiological evidence against the louse transmission of the disease in this country. His studies suggest that some other insect vector will probably be found, and there is considerable reason for believing that there is a natural reservoir for the disease in rodents, possibly rats or mice.

The cases reported by Brill were considered by many students of the problem to represent an importation of the disease from Europe, but the more recent work has shown that, at least in the south

* Received for publication January 14, 1931.

Atlantic states, the disease is endemic. On the whole, it seems more probable that Brill's cases were also of native origin.

The mortality from endemic typhus in this country is low and material for pathological study is therefore difficult to obtain. In 1911 Brill [4] described the autopsy findings of one case, and of two other probable cases. The pathology of typhus was not understood at that time and the brain (the most important organ from a diagnostic point of view) was not studied microscopically in any of these three cases.

Autopsy material from the fatal case to be reported here was obtained under ideal conditions, and a comparison of the pathology (in the human) of this American strain of typhus with that of European typhus was made possible. The pathology of European typhus has been studied in great detail by Wolbach, Todd and Palfrey [5] and the material on which their studies were based was available for purposes of comparison.

Report of Case

Clinical History: The patient was a white man, 53 years old, who lived about a mile west of Charlottesville, Virginia, in an isolated farm house. He worked as a dairyman at a nearby farm. For a month preceding the onset of his illness he had not been away from his home except for an occasional trip to Charlottesville. There was no history of contact with a preceding case and no known contact with any stranger of foreign origin. The man was the father of a large family and stayed at home most of the time. There were no secondary cases in the family or neighborhood, or in the hospital to which he was admitted without special precautions. He gave no history of lice or of other insect bites.

The onset of his illness was rather abrupt. On June 12 he developed a severe headache, joint and muscle pains, general malaise, nausea without vomiting, and fever. He was admitted to the hospital on the seventh day of his illness. At the time of his admission he appeared to be very toxic and it was impossible to get a coherent story from him. On the second day after admission a rash was noted over the trunk. This spread rapidly and soon involved the whole body. It was distinctly maculopapular in character and in the later stages became petechial. The eruption was so intense that it was plainly visible across the room. His temperature continued elevated, his pulse rapid, and his mental dullness deepened into coma. His condition became worse, respirations increased to a marked hyperpnea and he died on the eleventh day. On the final day of his illness the scrotum was examined and there was no apparent involvement of the testes.

Laboratory findings were as follows: The blood pressure was 170/100, hemoglobin 78 per cent, red blood count 5,400,000, white blood count 5,400, differential count within normal limits. The agglutination of Proteus X19 was negative on admission. The Wassermann reaction was negative. Lumbar puncture was

done on the day of admission and the findings were those of a normal fluid. The urine showed an occasional finely granular cast but no blood. The blood urea nitrogen was 112 mg. per 100 cc. Blood taken on the eleventh day after admission agglutinated Proteus X19 in a dilution 1:2560.

The clinical diagnosis was typhus fever.

Autopsy was performed nine hours after death by Dr. E. S. Groseclose.

Postmortem Examination

There was moderate emaciation, and the characteristic fine petechial eruption was present on the skin, especially on the hands, feet, buttocks and lower back.

The right lung showed dark red pneumonic consolidation of its lower lobe. Pleural scars were present at the right apex.

The right kidney contained an old white infarct on its lateral border measuring 2 by 1 cm. on cut surface. Otherwise the kidneys appeared negative.

The brain was markedly congested, rather soft and pinkish in color on section.

The peritoneal, pleural and pericardial cavities, heart, spleen, pancreas, liver, gall-bladder, gastro-intestinal tract, aorta and testes were essentially negative on gross examination.

The anatomical diagnoses were: early bronchopneumonia of the lower lobe of the right lung, healed tuberculosis of the apex of the right lung, old infarct of the right kidney and petechial eruption on the skin.

Microscopic Examination

The heart showed numerous lesions. Many of the precapillaries showed definite intimal proliferation and thrombus formation with perivascular accumulation of macrophages and lymphocytes. The thrombi were composed chiefly of endothelial cells and platelets, but in one instance eight or ten polymorphonuclears were present. A second type of lesion consisted of fusiform collections of cells between spread-apart, but not obviously damaged, muscle fibers. These cells included macrophages, endothelial cells, lymphocytes, plasma cells, mast cells and polymorphonuclears, their frequency being in the order mentioned. At first glance these lesions resembled focal necroses, but the muscle fibers did not appear damaged. On more careful study it was obvious that the great majority (and, by deduction, probably all) of these lesions had originated in the minute

capillaries of the heart wall by a process of endothelial proliferation, followed by a perivascular accumulation of macrophages and other cells. These lesions in the myocardium were entirely similar to those described by Wolbach, Todd and Palfrey in their European cases. There was moderate diffuse fibrosis of the myocardium, clearly an old process and not related to the acute illness.

The diagnosis of bronchopneumonia of the right lower lobe was confirmed histologically. Many of the alveoli contained only red blood cells and serum, and the infection had the appearance of a terminal one. In the uninvolved regions no lesions characteristic of typhus were present.

The spleen was moderately congested. The trabeculae and follicles were essentially normal in appearance and the pulp showed a predominance of mononuclear cells. The reticulo-endothelial cells were somewhat prominent. Occasional small collections of large mononuclear cells somewhat suggestive of typhus "nodes" were present and the venus sinuses in several instances appeared to be occluded by proliferation of their lining cells, but the structure of the spleen makes the recognition of proliferative vascular lesions almost impossible.

The liver showed accumulations of mononuclear cells in the portal areas and slight proliferation of the Kupffer cells, but the changes were such as occur frequently in other conditions.

The testes showed somewhat reduced spermatogenic activity which was not considered remarkable in view of the age of the patient. In the tunica albuginea, one group of adjacent small capillaries showed involvement of the type characteristic of typhus infection. There was no inflammatory reaction on the surface of the tunica.

The kidneys, pancreas and aorta showed no specific typhus lesions. Unfortunately no sections were made from the infarct in the kidney.

The most marked involvement was found in the brain. Of thirty-six sections studied, representing various regions in the cerebral cortex, basal ganglia, pons, cerebellum and brain stem, not one failed to show characteristic and even diagnostic lesions. These typhus lesions have been so carefully studied and described elsewhere that it is unnecessary to go into great detail. The earliest lesion is represented by small vessels which show prominence of the endothelium,

and occasional mitoses of the endothelial cells *in situ*. The lumen becomes obliterated by the proliferated endothelial cells and by the platelets and fibrin which collect as a result of damage to the lining of the vessel. Macrophages and cells of the lymphocytic series collect in the perivascular space, neuroglia eventually proliferates and the late lesion is a mass of closely packed cells of various types so that the vascular origin of the lesion eventually becomes obscured. Many vessels showed perivascular accumulations of mononuclear cells, some of which were in mitosis, without noticeable endothelial proliferation. In serial sections, however, the typical picture of endothelial proliferation was often found at some point along the course of such vessels. Large numbers of enormous phagocytic cells containing hemosiderin and chromatin inclusions were often seen in the perivascular spaces of the larger vessels, but the intima of these larger vessels was apparently normal.

Many of the thrombi contained numerous polymorphonuclears and pyknotic nuclear fragments. This feature was more marked than in the usual case of European typhus, and the lesions in the case reported here seemed unusually numerous and acute. Often there was a zone of vacuolization of the brain substance about these acutely thrombosed vessels, similar to that seen in an ordinary brain infarct. Another type of lesion seen in sections representing the internal capsule in this case was a small circumscribed area of hyaline degeneration of the white matter without cellular reaction. This appearance was apparently produced by agglomeration and fusion of small groups of damaged nerve fibers and probably represents paths of fiber degeneration, secondary to destruction of ganglion cells by involvement in the larger vascular lesions, although it may be of arteriosclerotic origin and unrelated to the acute illness.

Sections of skin showed typical vascular lesions which need not be described since they were similar to those in the heart and brain. Mast cells were a prominent feature of these lesions as in all typhus skin lesions.

The Demonstration of Rickettsia

Because of the fact that the brain had been fixed in formalin (which is not generally believed to be satisfactory for this purpose) it was thought that the demonstration of rickettsia would be a difficult task. The organisms were found relatively easily, however, and

were brought out so clearly in the cytoplasm of the proliferating endothelial cells that it is felt that the method used is possibly the method of choice for brain tissue. For this reason, the treatment which the tissues received will be given in detail.

The brain was fixed *in toto* in formaldehyde of the usual strength (4 per cent solution). One month later blocks 4 to 8 mm. in thickness were cut from various parts of the brain, placed in bottles of fresh formaldehyde of the same strength and sent to Boston. These blocks were then further cut to a thickness of 2 to 3 mm., washed in running water for 24 hours and then fixed for 36 hours in Regaud's fluid.*

After fixation the tissues were washed for 24 hours, dehydrated in graded alcohols, cleared in cedarwood oil and embedded in paraffin in the usual way. Sections were cut as thin as possible, and it is believed that this is a fairly important part of the technique.

The sections were stained overnight in Giemsa solution (2.5 cc. of concentrated solution, 2.5 cc. of methyl alcohol, 100 cc. of distilled water and 5 drops of 0.5 per cent sodium carbonate), changing the staining fluid twice during the first three hours.

The sections were stained a uniform deep blue and it was impossible to get satisfactory differentiation with colophonium or acetic acid, since both tend to decolorize the rickettsia before they produce color changes. Exposure to strong sunlight slowly differentiates the sections without much fading. The best results were obtained by carrying the differentiation to the point at which the red blood cells have turned from green to pink. The rickettsia at this stage stand out as deeply stained bluish purple structures in the faint blue cytoplasm of the endothelial cells. The majority of the organisms

* Regaud's fluid was first used (in a modified form) for the demonstration of rickettsia by Rosenberger.[6] Later it was used by Cowdry[7] in studying the rickettsia of heartwater. He gives the formula as 4 parts of 3 per cent potassium bichromate and 1 part of formaldehyde (40 per cent). The formula used here is that given in McClung's Handbook of Microscopical Technic:

Potassium bichromate	25 gm.
Sodium sulphate	10 gm.
Distilled Water	1000 cc.

Before using, add 20 cc. of 40 per cent (actual) formaldehyde to every 100 cc. of fixative.

For both human and guinea pig tissues, this has been found to be far superior to all other fixatives tested, and rickettsia are stained as sharply and as deeply as ordinary bacteria.

appear as definite rods, occasionally paired and rarely standing on end so that they resemble cocci. Definite coccoid forms were not seen, however.

With further exposure to sunlight, the organisms change to a light red color and then slowly fade. They may be restained and redifferentiated without loss of clarity. The apparent size of the organisms is greatly exaggerated when they are deeply stained, but as they differentiate and fade their true size becomes evident.

The organisms were found invariably and exclusively within the cytoplasm of the endothelial cells *in situ*, and were never definitely seen in the perivascular macrophages. They were not seen in neuroglia cells, ganglion cells, the cells which cover the meninges or those which line the ventricles. Many of the macrophages, especially in the later lesions, develop granular cytoplasm, but the granules cannot be recognized as organisms. In the very late lesions the entire background becomes a mass of minute blue-staining granules, often rod-shaped but never uniform in size and shape. Among these structures, purple-staining rickettsia in groups of 3 to 6 were occasionally recognized, but the majority of the granular structures were believed to be derived from broken up neuroglia fibrils. A similar appearance is often seen in the brain lesions of experimental typhus in the guinea pig, and a large number of sections have been prepared without ever bringing out these structures as definite organisms.

In several vessels as many as six endothelial cells were definitely observed to contain rickettsia. In many of the sections, by selecting under low power the earliest and most acute vascular lesions, it was possible to find rickettsia after a few minutes' search, but the majority of the lesions contained no demonstrable organisms. When found in a given lesion it was nearly always possible to find them in serial sections of that lesion mounted on the same slide.

The organisms were never very numerous, ranging from two or three up to eighteen or twenty in a single cell. They never packed the cytoplasm of the cells as they do in the gut of the louse and scrotal sac of the guinea pig.

Comment

The pathological changes in this case do not differ in any way from those described in the European series by Wolbach, Todd and

Palfrey. The rickettsia in the lesions also correspond morphologically to those described by these workers. The study has therefore confirmed both the essential similarity of American and European typhus and the etiological relationship of Rickettsia Prowazeki to typhus, although neither of these facts stood in serious need of confirmation.

The clinical differences between European and American typhus in the guinea pig are now quite well understood. Male guinea pigs inoculated intraperitoneally with American typhus virus react by a marked inflammation of the tunica vaginalis, and this reaction almost completely prevents the occurrence of the vascular lesions in the brain. For a time this fact caused confusion, in that it suggested a similarity of the disease to Rocky Mountain spotted fever, but the similarity is only apparent, since in the latter disease the reaction is primarily vascular and its occurrence is independent of the route of inoculation, while in typhus the reaction is chiefly in the lining cells of the tunica vaginalis and occurs only after intraperitoneal inoculation (Pinkerton [8]). When inoculation with American typhus material is done subcutaneously there is no reaction in the tunica, and brain lesions are frequently as numerous as in the European strain.

The absence of any inflammatory reaction in the tunica in the case reported here confirms the opinion already expressed that the tunica reaction in experimental infection does not represent a localization of the virus after gaining entrance to the blood stream, but is rather a reaction at the portal of entry when the artificial method of intraperitoneal inoculation is employed. The presence of numerous brain lesions in the reported case is what one would expect in view of the fact that natural inoculation in the human being is probably subcutaneous or intracutaneous and with relatively small numbers of rickettsia.

Summary and Conclusions

1. Study of a fatal case of endemic typhus in Virginia has established the complete pathological identity of the disease with European typhus.

2. Characteristic brain lesions were fully as numerous as in the average case of European typhus, and many of these lesions were unusually acute.

3. Involvement of the tunica vaginalis and scrotum was no more marked than in European typhus.

4. Rickettsia Prowazeki were easily and clearly demonstrated in the endothelial cells of the vascular lesions in the brain, but were not found in macrophages or neuroglial cells.

5. A technique is described for the demonstration of rickettsia in formalin-fixed brain material.

6. The demonstration of Rickettsia Prowazeki in this material is to be added to the already overwhelming evidence in favor of their etiological relationship to typhus fever.

NOTE: While this paper was in press our attention was called to the recent work of Badger, Dyer and Rumreich.[9] These workers have shown that two closely allied diseases occur in southeastern United States. In addition to endemic typhus, as described by Maxcy, they report the occurrence of a disease which they regard as more closely allied to Rocky Mountain spotted fever. We have considered the possibility that the case reported here belongs to the group of cases which these workers describe. In view of the fact that brain lesions have not been described in spotted fever, either in human or animal tissues, and because of the complete pathological identity of our case with European typhus, it is impossible to believe that it belongs in the spotted fever group. It is, of course, unfortunate that animal inoculation was not done from this particular case.

REFERENCES

1. Maxcy, K. F. The distribution of endemic typhus (Brill's disease) in the United States. *Pub. Health Rep., U. S. Pub. Health Serv.*, 1928, **43**, No. 47, 3084–3095.
Maxcy, K. F. Typhus Fever in the United States. DeLamar Lectures, 1928–29. The Williams & Wilkins Co., Baltimore, Md.

2. Brill, N. E. An acute infectious disease of unknown origin. A clinical study based on 221 cases. *Am. J. M. Sc.*, 1910, **139**, 484–502.

3. Anderson, J. F., and Goldberger, J. The relation of so-called Brill's disease to typhus fever. *Public Health Rep., U. S. Public Health Serv.*, 1912, **27**, No. 5, 149–160.

4. Brill, N. E. Pathological and experimental data derived from a further study of an acute infectious disease of unknown origin. *Am. J. M. Sc.*, 1911, **142**, 196–218.

5. Wolbach, S. B., Todd, J. L., and Palfrey, F. W. The Etiology and Pathology of Typhus. Harvard University Press, Cambridge, Mass., 1922.

6. Rosenberger, G. Studien über die in- und extrazellular liegenden Rickettsien. *Arch. f. Schiffs- u. Tropen-Hyg.*, 1922, **26**, 112.

7. Cowdry, E. V. Studies on the etiology of heartwater. II *Rickettsia ruminautum* (*n. sp.*) in the tissues of ticks transmitting the disease. *J. Exper. Med.*, 1925, **42**, 253–274.

8. Pinkerton, H. Rickettsia-like organisms in the scrotal sac of guinea-pigs with European typhus. *J. Infect. Dis.*, 1929, **44**, 337–346.

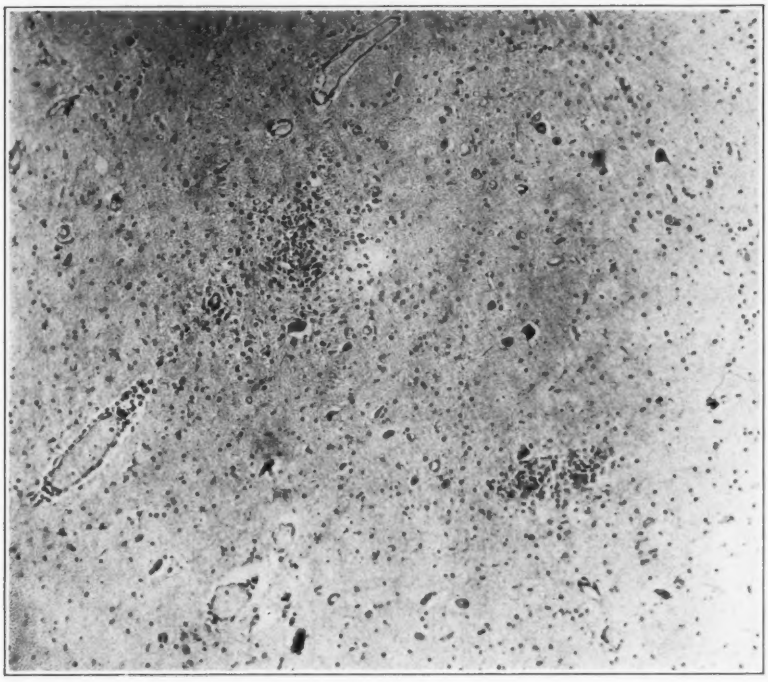
9. Badger, L. F., Dyer, R. E., and Rumreich, A. An infection of the Rocky Mountain spotted fever type. Identification in the eastern part of the United States. *Pub. Health Rep., U. S. Pub. Health Serv.*, 1931, **46**, No. 9, 463–470.

DESCRIPTION OF PLATES

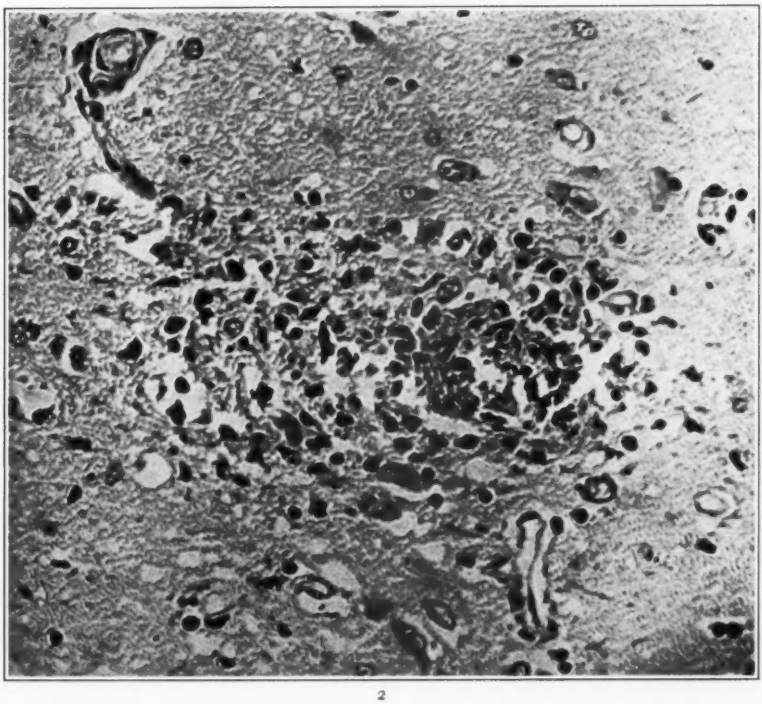
PLATE 21

FIG. 1. Low power view of vascular lesions in brain stem. × 80.

FIG. 2. A fairly late vascular lesion in the cerebral cortex. A suggestion of a central blood vessel is still present. × 700.



1



2

Pinkerton and Maxcy Study of Case of Endemic Typhus

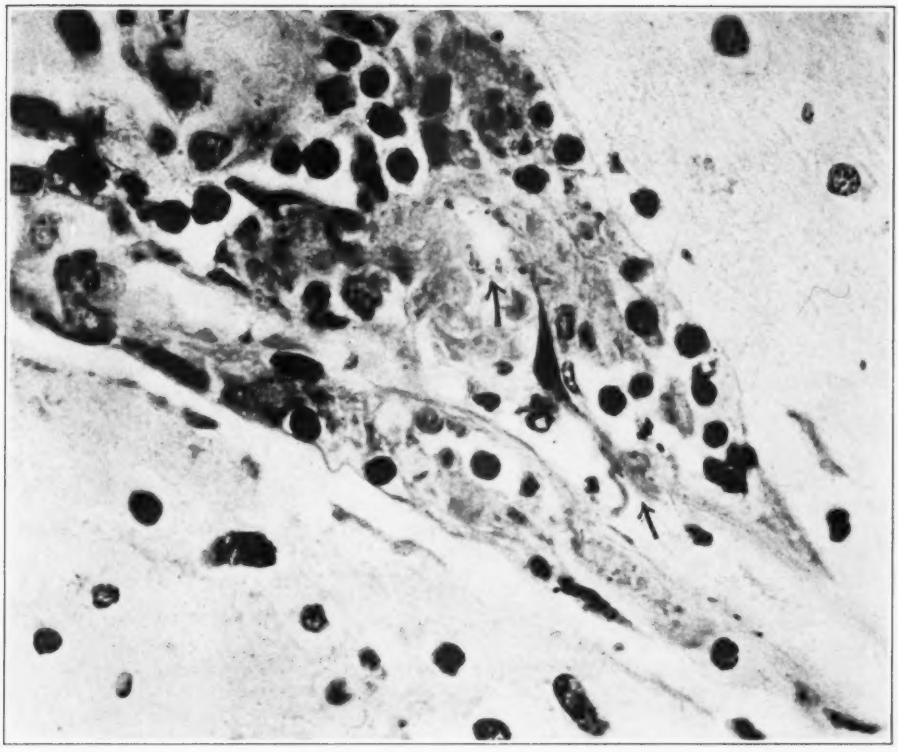
PLATE 22

FIG. 3. An acute vascular lesion in the cerebral cortex, showing early thrombus formation and perivascular infiltration. The arrows within the lumen point to endothelial cells containing rickettsia. × 1500.

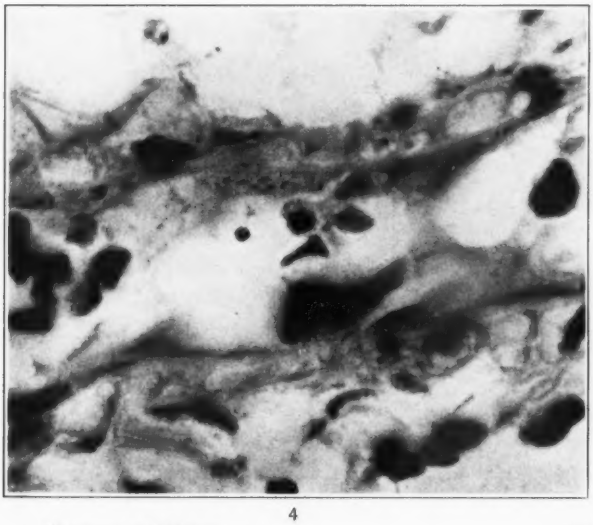
FIG. 4. An early lesion in the cerebral cortex showing rickettsia in upper endothelial cells. × 1500.

FIG. 5. Same cell as that shown in Fig. 4, focused at a different level. × 1500.

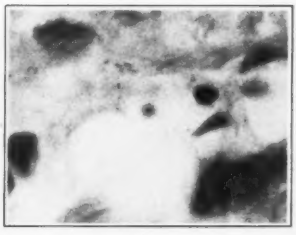
FIG. 6. Another group of rickettsia in same vessel as those in Figs. 4 and 5. One definite diplobacillus in a vertical position. × 1500.



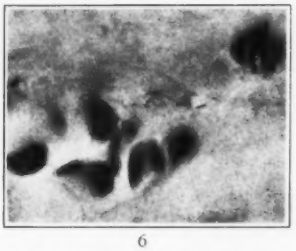
3



4



5



6

Pinkerton and Maxcy

Study of Case of Endemic Typhus

CONGENITAL RHABDOMYOMA OF THE HEART *

SIDNEY FARBER, M.D.

(From the Department of Pathology, Harvard Medical School, and the Pathology Laboratory of the Children's Hospital, Boston, Mass.)

Congenital rhabdomyomas of the heart are still considered rare tumors. Only two have been reported in the American literature, the last in 1907. Hence the finding of such a tumor, associated in addition with cerebral tuberous sclerosis, is deemed sufficient ground for this report. The increasing number of cases in the foreign literature in recent years affords also an opportunity for a review and consideration of the data now available. A search of the literature has been made, a few early cases, neglected by later writers, have been found and a few more have appeared as case reports since the last review.

CASE REPORT

Clinical History: E. B., a 6 month's old female infant, was admitted with a complaint of "pus in the left side of the chest." Her past and family history were irrelevant. The patient had always been apparently normal and well. The present illness began one month before admission when she began to vomit and had a high temperature. Physical examination showed evidence of pneumonia and empyema. The heart was enlarged and a harsh blowing systolic murmur was heard over the precordium. The neck was stiff and a bilateral positive Kernig sign was elicited. Death occurred nine days after admission and clinical diagnoses of pneumonia, empyema, meningitis, congenital heart and congenital anomaly of the kidney were made. A postmortem examination (A–30–28) was performed one hour after death.

POSTMORTEM EXAMINATION

Body: The body is that of a moderately well developed, poorly nourished, white, female infant, weighing 14 pounds and measuring 70 cm. in length. The circumference of the head is 42 cm. The anterior fontanelle is closed and the sutures are united. Rigor mortis is not present. There is moderate postmortem lividity over the dependent portions. The skin is dry, inelastic and hangs loosely over a small amount of subcutaneous fat. There are multiple, minute, purple, petechial hemorrhagic areas scattered over the abdomen. The hair is light in color, fine in texture and present in a moderate amount over the head. The ears and nasal passages are negative to external examination. The conjunctivae and sclerae are clear. The pupils are equal and regular, measuring 3 mm. in diameter. The hair is shaved from the right temporal region and several needle puncture marks are noted there as well as over the left jugular region,

* Received for publication January 14, 1931.

four above the seventh rib in the posterior axillary line, four over the same rib in the anterior axillary line, three over the vertebral column 1.5 cm. above a line even with the iliac crests, and one 1.5 cm. above the latter. There are linear abrasions over both buttocks. The cervical, axillary and inguinal lymph nodes are enlarged and firm to palpation. A cistern puncture is made and clear fluid under normal (living) pressure removed.

Primary Incision: The body is opened by the usual Y-shaped incision extending from both pectoral folds to the xyphoid process and thence to the symphysis pubis. There is a small amount of subcutaneous fat. The pectoral and abdominal muscles are brownish red in color. There is no diastasis of the recti muscles. A small, easily reducible umbilical hernia is present. The umbilical vein and hypogastric arteries are not patent, and repeated sections show no evidence of inflammation.

Peritoneal Cavity: There are about 100 cc. of clear straw-colored fluid. No adhesions are present. The liver edge extends 6.5 cm. below the right costal margin in the midclavicular line. The spleen extends 3.5 cm. below the left costal margin in the midaxillary line. The intestinal loops are distended. There is no noticeable abnormality of the mesenteric attachments. The mesenteric lymph glands are firm, injected, and slightly enlarged, the average measuring 0.5 cm. in diameter. The appendix is retrocecal in position and is coiled upon itself, measuring 5 cm. in length. The dome of the diaphragm extends to the fifth rib on the right and the sixth rib on the left. There are no adhesions between the liver and diaphragm. The kidneys can be palpated through the peritoneum and appear enlarged and displaced downward. The bladder is empty and contracted. The uterus, ovaries, Fallopian tubes, broad and round ligaments do not appear unusual.

Pleural Cavities: In removing the sternum a well encapsulated circumscribed empyema cavity on the left is accidentally entered. The medial margin of this cavity is 2 cm. to the left of the midsternal line adjoining the outer surface of the pericardial sac for a distance of 4 cm.; its inferior boundary is the diaphragm for a depth of 2 cm.; its superior margin is a strong fibrous wall attached along the chest wall in the midaxillary line as far up as the second rib, and its lateral wall is the inner chest wall. Smears of the creamy yellow seropurulent contents show Gram-positive cocci in long chains. This cavity shows no evidence of communication with the external surface, nor with another larger cavity which contains similar contents and is situated to the left and immediately beneath it. This cavity has many finger-like connecting vestibulae and measures roughly 5 cm. in diameter. The consolidated left lung is pressed closely to the mediastinum. There is evidence of connection between this second cavity and the external surface of the body through the needle punctures mentioned previously. The lower lobe of the left lung above this latter cavity is firmly bound to the chest wall by multiple fine, fibrous adhesions. The pleural surfaces on the left are rough and covered with a shaggy yellowish fibrinopurulent exudate. Those on the right present a marked contrast. They are clear, smooth and glistening and show no evidence of adhesions.

Mediastinum: The thymus weights 9 gm. There is no apparent displacement of the mediastinum. The lymph nodes are firm, slightly enlarged and injected. The right innominate, left carotid and left subclavian vessels are given off in their usual order.

Pericardial Cavity: Contains a small amount of clear straw-colored fluid. Smears show no organisms.

Heart: Weighs 90 gm., (normal weight 31 gm.). It measures 6 cm. in its transverse, and 5 cm. in its longitudinal diameter. The epicardium is smooth and glistening and there is no evidence of hemorrhage. There is a bulging mass over the interventricular septum near the auricular ventricular extremity and on closer examination a small, raised circular circumscribed area measuring 1 by 0.2 cm. is located 1.5 cm. from the right lateral margin of the pulmonary artery orifice. The anterior margin of the artery is on a line with the posterior margin of the protuberance. The epicardium does not appear to be interrupted. On opening the right auricle the valve orifice presents a bulbous, yellowish white, firm encapsulated mass 0.8 cm. by 0.9 cm. which projects 2 mm. above the valve ring into the right auricle. A probe can be passed between the cusps and the mass on all aspects except the angle between the anterior and left posterior lateral cusp where the mass is firmly attached and shows evidence of early necrosis, manifested by a dark brown color and softening. There are two small, slightly raised yellowish white areas on the anterior cusp in the midportion, each measuring 2 mm. in diameter. One other area of similar nature is located above the valvular attachment, attached to the auricular wall. On opening the right ventricle, similar masses can be found on the left lateral cusp in close apposition to the larger bulbous mass which is now seen firmly attached to the ventricular wall immediately below the angle of the anterior and left lateral cusps. Its total height is 13 cm., the diameter 0.8 to 0.9 cm. Multiple dome-like, firm, yellowish pink, glistening, smooth projections standing about 2 mm. each above an otherwise grayish white endocardium along the posterior surface of the ventricle up to the pulmonary valve are seen. The largest one on this surface measures 7 mm. On palpation this seems merely to be the surface manifestation of a larger firm mass beneath, which is continuous with the base of the first described bulbous mass. The smaller ones averaging 2 mm. in diameter and projecting about 2 mm. above the surface are grouped near the pulmonary valve orifice where they form a cauliflower-like mass firmly binding the left posterior and right posterior valve leaflets to the heart wall. Several similar small excrescences may be seen between the papillary muscles near the extreme apex of the right ventricle. A large conglomerate mass similar to those described above and measuring 1.5 cm. in diameter is located on the anterior ventricular wall just above the larger papil-

lary muscles supporting the anterior cusp of the tricuspid valve. This fits into the space left by the two larger growths previously described, thus causing partial obstruction to the blood flow from the right ventricle to the pulmonary artery. The left auricle is uninvolved. A few smaller areas, approximately 1 mm. in diameter, may be seen scattered throughout the myocardium of the left ventricle, and one small mass measuring 1 mm. in diameter can be seen just below the junction of the two lateral aortic cusps. On cutting into the circular raised area on the interventricular septum from the epicardial surface a yellowish, moderately firm, smooth, slightly bulging surface is revealed. On further section this proves to be only a small cap of a sharply defined tumor mass measuring 3 cm. in diameter. This is grayish yellow in color and firm in consistency. There is a suggestion of a concentric arrangement near the line of gradual merging with normal-appearing myocardium. The myocardium itself is brownish red in color and shows no other abnormality. The thickness of the left ventricle is 7 mm., that of the right 5 mm. The auricles measure 2 mm. in thickness. The valves measure, roughly: tricuspid valve 5 cm., pulmonary valve 2 cm., mitral valve 3.5 cm. and aortic valve 3 cm.

Lungs: Right lung weighs 80 gm., the left 110 gm., (normal weight 42 and 38 gm. respectively). The right lung presents a normal yellowish pink appearance and crepitant lappets partially cover the pericardium. The posterior portion of this lung, however, is deep purple in color and firm in consistency, with a mottled appearance given to it by intervening lighter portions. The cut surface here reveals a deep red surface, dry and granular in appearance with thickened bronchioles standing out prominently. The anterior portion exudes a frothy pinkish exudate on pressure.

The left lung is firm, dark red in color and covered with a shaggy yellowish fibrinopurulent exudate. A section near the apex reveals two small circumscribed abscess cavities measuring 1 cm. in diameter. Other sections show marked thickening of the bronchioles, causing them to stand out as white spots against a deep red, meaty, airless background.

Spleen: Weight 50 gm., (normal weight 17 gm.). Deep purple in color and slightly less firm than normal. The capsule shows no evidence of thickening or inflammation. The cut surface reveals a deep purple, soft background, against which multiple prominent splenic corpuscles form an evenly distributed speckled contrast. Thin sections are friable and do not retain their shape.

Gastro-Intestinal Tract: Opened from the esophagus to anus. The mucosa of the esophagus and stomach does not appear unusual. There is a small amount of bile-stained material in the duodenum. The jejunum, ileum, descending and transverse colons present rare, minute, purple hemorrhagic areas. There is no evidence of gross blood. Peyer's patches do not appear unusual. There are no gross anomalies.

Pancreas: Normal in shape, size and consistency.

Liver: Weight 350 gm., (normal weight 200 gm.). It is mottled yellow and purplish red in color. The capsule is not thickened. Cut section shows a fine streaking of congested vessels forming a network for radiating yellowish white linear areas against a yellowish red background. Uniformly distributed, minute grayish spots add focal necrosis to the possibilities of fatty infiltration and passive congestion.

Gall-Bladder: Moderately distended with clear yellow bile which is easily expressed through the ampulla of Vater without difficulty. The mucosal surface is smooth and velvety. The duct system presents no abnormalities.

Kidneys: Right weighs 60 gm., left 70 gm., (normal weight 26 and 25 gm. respectively). They are mottled, yellowish red to grayish red in color, with darker areas which bulge slightly above the surface, distributed irregularly. These areas are fluctuant and vary in size from 1 mm. to 7 mm. The capsules strip with ease. The parenchyma everts on cutting. The cortex and medulla are well defined, but irregular. The medullary pyramids in some areas are displaced by firm, yellow, granular-appearing material. Several cystic areas are encountered which contain clear straw-colored fluid. The pelves are smooth, white and glistening. There is no evidence of uric acid deposits. The ureters are of average caliber and pursue their usual course to the bladder.

Adrenals: Normal in size, shape and location.

Genitalia: The external genitalia are normal. The uterus, Fallopian tubes and ovaries are in their normal location.

Aorta: Opened throughout its length it reveals no evidence of atheromatosis or degeneration.

Organs of Neck: The thyroid appears normal in size, shape and consistency.

Bone Marrow: The osteochondral junctions are even and regular. The line of provisional ossification is not widened. The bone marrow itself is deep red in color.

Spinal Cord: Removed from the cervical to lumbar region. There is no evidence of extra- or subdural hemorrhage.

Brain: Weight 780 gm., (normal weight 659 gm.). The usual mastoid to mastoid incision is made and the scalp flaps reflected. The superficial fascia and galea aponeurotica present no unusual features. The bony structure of the head is normal. There are no exostoses and no evidence of cranio tabes or osteoporosis is present. The calvarium is removed by sawing through the skull in the occipitofrontal circumference and separating it from the dura. The internal structure of the skull is normal. There are no hemorrhages involving the dura which is cut in the same line with the skull and reflected medially. The falx and tentorium are intact with no evidence of old or recent hemorrhage. The vein of Galen is not thrombosed and presents its normal appearance. The brain is removed by cutting the nerves and other attachments in order. On examination

after removal a marble-like pallor is the only unusual feature noted by inspection. The convolutions have the normal appearance; the sulci are of the usual depth. There is no congestion or thrombosis of the vessels and no areas of hemorrhage. The meninges are slightly less translucent than normal, but show no other evidence of inflammatory reaction. The cerebrospinal fluid is normal in character and amount. On palpation there is no undue fluctuation. As the hand is passed lightly over the brain a remarkable uneveness of resistance is discovered. Areas of normal consistence are adjacent to others of stone-like firmness. The distribution of these areas of sclerosis follows no definite pattern except that they are more or less limited by the sulci, rather than spreading in a diffuse manner over portions of several convolutions. Very slight variation in the degree of firmness exists. A few areas of sclerosis may be felt beneath a surface covering of normal cortex. The sclerosis is not more marked on any one part of the brain. The cerebellum and midbrain are not involved and are of apparently normal consistence and structure. After formalin fixation nothing further is found to add to the previous description of the external surface. Two longitudinal sections are made 0.5 cm. to each side and parallel with the median longitudinal fissure. Gray and white matter are well differentiated except in certain areas, which on palpation are extremely firm and found to be continuations of the external areas of sclerosis. These firm cortical areas have either the uniform appearance of white matter or a slightly grayish tinge shading off into white with no definite demarcation. In the white matter of the frontal lobe there is a cluster of firm areas 2 to 3 mm. in diameter. Similar firm areas 1.1 to 0.4 cm. in diameter project from the corpus striatum into the lateral ventricles. No areas of hemorrhage or softening are present. The internal structure of the brain is normal and the ventricular system of the usual size. There is no evidence of ventriculitis or choroiditis. The cerebellum shows no evidence of sclerosis.

Sinuses: The venous sinuses are explored. No ante mortem thrombi are found. The endothelial surfaces are smooth and glistening. The dura is stripped from the base of the brain. The bony structure of the base of the skull is normal.

Middle Ears: Opened in the routine manner. A thin, yellow purulent fluid is found in both middle ears, which on smear shows Gram-positive cocci in chains. The tympanic membranes are slightly opaque but intact. A slight amount of the same fluid is found in both mastoids but there is no evidence of bone necrosis.

Anatomical Diagnoses: Congenital rhabdomyoma of the heart, cerebral tuberous sclerosis, congenital polycystic kidneys, meningitis, bronchopneumonia, lung abscess and empyema due to streptococcus hemolyticus.

Microscopic Examination

Heart: After the heart was partially sectioned, vertical slices, approximately 2 mm. in thickness, were taken through the large tumor in the interventricular line, from a smaller tumor nodule in the right ventricle, and from an area in the right myocardium, apparently free from tumor. These were fixed in Zenker's fluid. A thin section from the largest nodule was fixed in absolute alcohol and a similar section was teased on a slide and examined immediately. The Zenker-fixed material was stained by the following methods: hematoxylin and eosin, phosphotungstic acid hematoxylin, Mallory's connective tissue stain (phosphomolybdic acid, anilin blue, orange G after acid fuchsin), and Van Gieson's method. The section fixed in absolute alcohol was stained by Best's carmine stain for glycogen.

The two sections taken from the tumor nodules show little variation. There is no trace of normal tissue. Under low power numerous large vacuolated spaces varying from round to oval in shape and irregular in size dominate the picture, giving to the sections a loose, sponge-like appearance. Numerous heavy connective tissue trabeculae, in which lie blood vessels, course through the tumor, giving off finer and more delicate branches which ramify among the large vacuolated spaces. Thick protoplasmic walls surround the spaces and between them in places a thin, delicate connective tissue reticulum can be found. This can best be seen with the connective tissue stain. There is, however, no relation between this connective tissue reticulum and the walls of the vacuolated spaces.

Some of the spaces are empty. Others show cells with many processes lying within the clear spaces. The cells lie usually in the center of the spaces, or, less often, against the wall. There is great variation in the size and shape of the cells, depending in part on the size of the space within the cell. The processes run from the center protoplasmic mass in bizarre and irregular fashion. Sometimes they anastomose richly and divide into finer elements until they merge with the wall. Often the processes are short, plump and few in number, in

places failing to merge with the wall. On closer examination it is seen that these processes are transversely striated by rows of delicate granules. With suitable stains these granules stand out clearly, and the processes as well as the walls of the spaces show numerous definite cross-striations. The location of the spaces within the cells, as described by previous observers, is noted.

The cells generally have but one nucleus, occasionally two, and rarely three and four nuclei are found in a single cell. Sometimes clear spaces surround the nucleus and in such cases the processes are generally short and plump. A single nucleolus is usually present and occasional evidence of direct division is noted. No mitotic figures are seen.

The sections were compared with a specimen of the tumor described by Wolbach [1] and found to resemble it so closely in both the size of the elements and the finer histological details, that the following description is taken from Wolbach's paper and given freely as follows:

"The tumor is composed largely of cells with striated fibrils, in some instances with the full detail of normal fibrils, in most instances in the form of granules united by delicate fibrillary material. The granules stain deeply with iron hematoxylin. With phosphotungstic acid hematoxylin the granules stain a deep blue, the fibrils pale brownish or gray. With Mallory's connective tissue stain the granules stain red, the fibrillary substance blue.

Cells showing least structural detail contain clusters of paired granules having the staining properties of centrioles, and like centrioles are embedded or connected by centroplasm or centrolin. Individual granules (centrioles) are in the neighborhood of 0.2 microns in diameter. Larger bodies are probably clusters of unresolvable granules. The granule clusters rarely are as large as 2 microns in diameter; 1 to 1.5 microns diameter are frequent, but the majority are 0.5 to 1 micron.

Next in order of complexity are cells with great masses of paired granules. Other cells show a few fine fibrils connecting the granules and the beginning of an orderly arrangement in process toward the formation of the segmented fibril. The most completely matured cells are filled with segmented fibrils in bundles running in all directions. A few contain fibrils with alternate broad and narrow cross-striations but most of the fibrils have the appearance of granules strung on fibrils uniformly apart at distances of 1.6 microns and arranged in phalanx formation. However, scattered among the segmented fibrils are clusters of centrioles composed of from two to many members, often in diploid form. These centriole clusters have all the characteristics of those in the least differentiated cells and may be interpreted as clusters, the members of which have failed to disperse.

The tumor cells vary from 50 to 200 microns in diameter. The majority of the nuclei lie between the limits of 9 by 7 microns and 13 by 10 microns."

The third Zenker-fixed section, taken from the right myocardium, showed essentially normal myocardium, except for two widely separated islands of tumor cells. These consist of from four to eight vacuolated spaces, surrounded by zones of dense connective tissue. The spaces are empty and the walls show definite cross-striations. These small isolated areas in the myocardium are similar to those described by Rehder.[25] The sections stained by Best's method show large amounts of glycogen in the form of fine droplets filling the spaces and gathered thickly along the cell processes.

Spinal Cord: The nerve cells stain fairly well; however, several of the nuclei are eccentrically located. Nissl bodies stain darker than the nuclei and are located near the periphery in all cases. The glial cells are present in about their usual proportion. There is no evidence of gliosis, hemorrhage or areas of softening. The meninges are not thickened and there is no evidence of exudate.

Brain: There are fifty sections representing every part of a complete sagittal section 0.5 cm. to one side of the longitudinal fissure. The sections may be divided into those showing sclerosis grossly and those from apparently normal areas. There is a chronic and acute meningitis evident in both sets of sections. The meninges are slightly thickened by fibrous tissue and organizing exudate. Polymorphonuclear leucocytes occur in rather large clusters in more or less isolated areas of the meninges, chiefly but not exclusively around the meningeal vessels. Plasma cells and macrophages are fairly numerous. The reaction extends to a slight extent along the pia and is found in the perivascular spaces of the small vessels in the nervous tissue. A few cocci in pairs and short chains are occasionally seen among the polymorphonuclear leucocytes. Several of the capillaries are entirely filled with cocci, indicating postmortem growth.

The sections from the sclerosed areas show three distinct types of reaction. First, there is a marked gliosis forming a dense network of fibrils with fewer nuclei than are seen in normal areas. The increase in the fibers of the tangential network is more prominent than that in any other areas, with the exception of the sclerosed projections of the corpus striatum into the ventricles, which are covered with a similar dense network. There are different degrees of gliosis shading off almost imperceptibly to normal. Second, there are areas of less marked gliosis but with many immature glia cells of very large size with many fine spider-like processes and one to three small oval

nuclei. These cells are somewhat more numerous in the deeper layers of the cortex among the large pyramidal cells and polymorphous nerve cells. The third type of reaction indicates a secondary calcification with many deep blue calcified areas, some of which are slightly laminated. In the areas of sclerosis a few nerve cells are seen, but they are smaller than normal, have a distinctly compressed appearance and stain a deep blue. Edema is a prominent feature in all the sections but appears to be more marked in some of the areas of sclerosis than in others. The nerve cells and many of the large glia cells are surrounded by wide clear spaces which are traversed by nerve fibers or delicate glia fibrils. The perivascular spaces are also considerably wider than normal and many of the capillaries are narrow and appear compressed. The sections from portions with no gross pathology are normal except for moderate edema and chronic meningitis already described. In one section there is slight evidence of choroiditis, but no ventriculitis.

Review of Literature and Discussion

Only thirty-three undoubted cases (exclusive of our own) of the multiple type and eight of the single type of rhabdomyoma of the heart have been reported. In addition, one case of replacement of the entire myocardium by a diffuse rhabdomyoma [2] has recently been published, thus making a total of forty-one cases already on record.

The earlier cases of Kantzow and Virchow,[3] Skrzeczka,[4] Rieder,[5] and Justi,[6] rejected in the papers of Seiffert [7] and Wolbach,[1] were again reviewed and likewise rejected. The case of Kantzow and Virchow proved to be hyperplasia in the region of a gummatous process in the heart. Skrzeczka's case showed so much postmortem change that recognition was impossible. Rieder's case was regarded by Weigert as a true congenital malformation and not as a tumor. The description given by Justi was too poor to permit judgment.

Kennard [8] recently reported the finding, in a man 41 years of age, of a primary tumor of the heart associated with porencephalus. The heart weighed 750 gm. The tumor was situated in the apex and involved the wall of the left ventricle. Kennard's histological description does not include the few characteristic details which have been repeated with almost monotonous regularity by every author.

Hence, his case will not be included in this series. Bradley and Maxwell [9] recently placed on record a tumor which they believe is the first of its kind to be reported. The patient was a man of 62 years who had complained of pain in the upper left chest for five to six weeks before death. A tumor mass was found in the pericardial sac, in the heart wall and in the mediastinum. The heart and mass weighed 1900 gm. The brain was not examined. They believed that they were dealing with a rhabdomyosarcoma, primary in the heart. Their microscopic description does not correspond to the other accepted cases, and the metastases alone serve to set this case apart from those discussed here.

Three cases, about which no details could be obtained but which appear to be reliable, are included in a separate section of the accompanying table. Askanazy [10] in a discussion following Seiffert's report of his second case,[11] stated that he had investigated a case in which he had found glycogen. This case he apparently has not published. Goldstein [12] mentioned a case seen by Lucke at the Blockley Hospital. No details accompany the statement. Steinbiss [13] described the microscopic specimen of an incorrectly diagnosed heart tumor which came to his attention. This he had no hesitancy in calling a typical rhabdomyoma. Since Steinbiss recorded six other cases and described with great accuracy the histological details, this accidental finding should be listed as an additional unclassified case.

One case in animals [14] has been reported. Multiple rhabdomyomas were found in the heart of a well developed 6 month's old female pig. The brain and kidneys were not microscopically examined, but they were grossly negative.

Wolbach mentions in his paper, but does not include in his table, a case reported by Billard [15] which occurred before the days of histological examination. There were three small tumors in the anterior wall of the left ventricle and in the interventricular septum of an infant, 3 days of age. Billard's case was mentioned by Kolisko [16] but omitted by Seiffert. Wolbach thought that Billard's case was probably one of multiple rhabdomyomas.

Seiffert's second case was reported a few months after his first, and was neglected by all later writers. The inclusion of this case in the table, as well as complete tabulation of all authentic cases, will account for the discrepancy in the total number of cases as compared with previous reviews.

The apparent rarity of this tumor demands some explanation. A number may be missed in the absence of routine microscopic examinations. Steinbiss offers a somewhat ingenious explanation. Since so many of these cases are associated with tuberous sclerosis, and since it is now known rhabdomyomas of the heart are not confined to the first few years of life, he argues that the highest incidence would probably occur in institutions for the care of the feeble-minded. His own experience seems to bear that out, for in ten years in such an institution Steinbiss found twenty-one cases of tuberous sclerosis, six of which were associated with rhabdomyoma of the heart. If, in such institutions, more autopsies were done and if of these fewer were limited to the brain, more cases of rhabdomyoma of the heart might be found.

Most of the earlier reported tumors occurred in individuals under 3 years of age, and that observation was frequently stressed as proof of their congenital nature. A survey of the accompanying tables shows that twelve of the forty-one undoubted tumors were found in patients over 3 years of age, the oldest being 35 years. The sex is predominatingly male, but the series is naturally too small to permit any conclusion. Poor nutritional states were found in many instances, and several early authors thought of some relation between the poor nutritional state and the heart and brain pathology. A few patients, however, were in an excellent state of nutrition. Several showed marked physical retardation (Jonas,[17] Steinbiss [13]). The tumor described by Jonas was in a 6 month's old male infant who weighed 3.5 Kg., and measured 55 cm. in length, approximately a little more than the measurements and weight of a normal full-term infant. The third case of Steinbiss was a 10 year old male who weighed 10 Kg., and had no teeth.

The nature and location of the large spaces in the microscopic picture were for a time a subject of debate. Von Recklinghausen,[18] who described the first case, arrived at no conclusion, but thought that they might be lymph spaces (similar to the macroglossia of Virchow), blood spaces or muscle tubes of pathological origin. Virchow [19] stated "whether we shall regard the cavernous spaces as lymphatic or just as serous cavities, cannot be settled. The main substance of the tumor was muscular, however, and apparently a result of hyperplasia of the myocardium." Hlava [20] regarded the spaces as artefacts due to the effect of alcohol fixation and placed their position as in-

tracellular. Kolisko [16] believed the spaces lay between cells and so located them in a plate accompanying his paper. Cesaris-Demel [21] thought the spaces were intercellular, similar to spaces he found between anastomosing cells in the hearts of human embryos. Seiffert [7] was the first to produce embryological evidence to locate the spaces. Ponfick [22] and Knox and Schorer [23] agreed that the spaces were intracellular in position but offered no additional proof. The evidence of Seiffert was convincing, but it remained for Wolbach, with the aid of the phosphotungstic acid hematoxylin stain, to offer conclusive evidence of the intracellular position of the spaces.

Cesaris-Demel was the first to call attention to the many-processed cells lying within the spaces and these he called spider-like cells. Seiffert likened the cells to spiders in their webs, and the characteristic cell of this tumor has since been called the "spider cell." Steinbiss objects to this designation and suggests that a more related comparison would be more suitable. He compares the tumor cells to cells of Tradescantia. Running from the primordial tube which lies beneath the cell membrane are plasma threads which anastomose richly with one another until they unite in a larger plasma mass near to the center, leading to the nucleus. Between the threads are vacuoles filled with fluid. This he uses to further the comparison by mentioning the glycogen-filled vacuoles in the tumor cells.

The contents of the vacuolated spaces were not definitely recognized by the earlier writers. Although some looked upon them as evidences of degeneration, Marchand,[24] in a discussion to Seiffert's second paper (1900), reasoning by analogy to fetal muscle, suggested that glycogen might be present. In the same discussion Askanazy stated that he had demonstrated glycogen in small droplets in a case which he had occasion to study. Although Seiffert had been unable to demonstrate glycogen clearly, he was convinced that it was present in the spaces. In 1914, both Rehder [25] and Mönckeberg [26] were able to demonstrate glycogen in the tumor cells. Later writers also reported a similar finding, and I was able to find glycogen in large amounts in my case. Bundschuh [27] found small amounts of fat in fine droplets in the muscle cells at the periphery of the tumor. Kawamura [28] described fat in small and large droplets in the vacuoles and fibrils and Mittasch [29] mentioned that his cases showed both fat and glycogen. Steinbiss stated definitely that the vacuoles were free from fat. Walbaum [30] could find no fat in the myocardium of fetuses and young infants.

TABLE I

Multiple Forms in Cases of Rhabdomyoma of the Heart

Author	Heart	Brain	Other Organs	Age	Year	Remarks
1. von Recklinghausen [18]	Nodules in both ventricular walls	Tuberous sclerosis	Negative	Newborn	1863	
2. Virchow [19]	Numerous nodules in both ventricular walls	Not mentioned	Liver large. Multiple tumors in skin	Newborn	1864	Well nourished. Omphalitis
3. Kolisko [16]	Small nodules in right conus arteriosus and in anterior wall of right ventricle and septum	Negative		2 mos.	1887	Found dead
4. Cesaris-Demel [21]	Large tumor in apex of right ventricle. Many small nodules in left ventricle wall and septum	Tuberous sclerosis	Small nodules of embryonic renal tissue without glomeruli	3 yrs.	1895	
5. Seiffert [7] (Case 1)	Large tumor at apex, small nodules in myocardium and septum	Negative	Kidney cysts	20 mos. (male)	1900	Rickets
6. Seiffert [11] (Case 2)	Multiple small tumors in myocardium	Not mentioned	Not mentioned	7 mos.	1900	Rickets
7. Rothe [42]	Multiple tumors. No details	Tuberous sclerosis	Multiple tumors of breast	?	1901	
8. Ponfick [22] (Case 1)	Large nodules in right ventricle, nodules in both ventricular walls	Tuberous sclerosis	Not mentioned	7 mos. (male)	1901	Bronchopneumonia. No psychic disturbances
9. Ponfick (Case 2)	Large nodule in anterior wall of right ventricle, numerous nodules in left	Tuberous sclerosis	Not mentioned	3 yrs. (female)	1901	Chronic furunculosis. Emaciation

10. Bonome-Cagnetto [42]	Multiple tumors. No details	Tuberous sclerosis	Not mentioned	18 mos.	1895	Rickets
11. Knox and Schorer [23]	Large pedunculated tumor in left ventricle. Numerous small ones	Not mentioned	Negative	7 mos.	1906	Malnutrition
12. Abricossoff [22]	Multiple nodules in both ventricular walls. Two large nodules in anterior wall of left ventricle	Tuberous sclerosis	Negative	3¼ yrs.	1909	Died fourth day of scarlet fever
13. Kaufmann [44] (Case 1)	Multiple nodules	Tuberous sclerosis		3 yrs.	1922	
14. Kaufmann (Case 2)	Multiple nodules	Tuberous sclerosis	Multiple kidney tumors	7 yrs.	1922	
15. Schulgin [31] (Case 1)	Multiple tumors in both ventricular walls	Tuberous sclerosis	Kidney tumors	6 days	1913	
16. Schulgin (Case 2)	Multiple tumors in both ventricular walls	Tuberous sclerosis		6 yrs.	1913	
17. Bundschuh [27]	Nodules in left ventricle, apex and septum. One nodule composed mainly of fat	Tuberous sclerosis	Kidney tumors and cysts. Adenoma sebaceum	2 yrs. (female)	1912	Well nourished. Convulsions
18. Kawamura [28]	Multiple nodules in both ventricular walls and especially in septum. One in left auricle	Tuberous sclerosis? Brain not examined	Kidney tumors. Congenital anomalies in pancreas, esophagus and rectum	4 yrs. (female)	1913	Idiot. Epileptiform convulsions
19. Jonas [17]	Multiple nodules in both ventricular walls. Small nodule in right auricle	Tuberous sclerosis	Hare lip. Cleft palate	6 mos. (male)	1912	Poorly nourished, underweight, underdeveloped

TABLE I (*cont.*)

Author	Heart	Brain	Other Organs	Age	Year	Remarks
20. Mönckeberg [26]	Multiple nodules, especially at points of attachment of mitral and tricuspid valves	Firm consistency of cortex. (No microscopic examination)	Absence of right kidney, ureter and vessels	14 mos.	1914	Symptoms of "brain affection" before death. Recovering from measles
21. Rehder [25]	Large nodule at apex, reaching to septum. Small ones in right ventricular wall. Pulmonary artery obstructed	Grossly negative. (No microscopic examination)	Grossly negative	Newborn	1914	Well developed and nourished
22. Ribbert [28]	12 nodules in myocardium	Tuberous sclerosis	Multiple kidney cysts	1 yr.	1915	Died of diphtheria
23. Mittasch [29] (Case 1)	Nodule in right auricle. Numerous nodules in lower third of median wall of conus arteriosus and in lower half of auricular septum	Tuberous sclerosis	Kidney cysts and nodules of undifferentiated renal tissue	4 mos.	1922	Measles, pneumonia
24. Mittasch (Case 2)	Multiple nodules. No details	Tuberous sclerosis	Kidney tumors	14 yrs. (male)	1922	Acute yellow atrophy
25. Mittasch (Case 3)	Multiple nodules. No details	Tuberous sclerosis	Kidney tumors. Angiomyolipoma of liver	31 yrs. (male)	1922	Pneumonia and sepsis
26. Steinbiss [12]	Nodules in left ventricular wall, septum and papillary muscle of right ventricle	Tuberous sclerosis	Fibro-epithelioma of skin over face	5 yrs. (male)	1923	Idiot. History of epileptiform seizures

27. Steinbiss (Case 2)	Numerous nodules, especially in right ventricular wall. Larger nodules at left apex	Tuberous sclerosis	Kidney tumors	8 yrs. (male)	1923	Epilepsy. Death in seizure
28. Steinbiss (Case 3)	Multiple nodules in both ventricular walls	Tuberous sclerosis	Kidney tumors. Adenoma sebaceum	10 yrs. (male)	1923	Idiot. Death in marasmus. Weight 10 Kg. No teeth
29. Steinbiss (Case 4)	Nodule in septum and cicatrix-like nodule in left myocardium	Tuberous sclerosis	Kidney tumors and cysts	16 yrs. (male)	1923	Idiot. Death in seizure
30. Steinbiss (Case 5)	Two pea-sized nodules in right auricle	Tuberous sclerosis	Kidney tumors	21 yrs. (female)	1923	Mental deterioration since puberty
31. Uehlinger [38]	Numerous nodes in left myocardium septum. Large node at foot of anterior papillary muscle	Leptomeningitis. Chronic fibrosa. (No microscopic examination)	Negative	20 yrs. (male)	1925	Tetanus
32. Riedmatten [51]	Multiple nodules	Tuberous sclerosis		1½ yrs.	1904	
33. Berger and Vallée [50]	Multiple nodules	Brain not examined	Polycystic kidneys	2 yrs.	1930	Epileptic. Well nourished
34. Farber	Multiple large and small masses chiefly in right ventricle	Tuberous sclerosis	Polycystic kidneys	6 mos. (female)	1930	Died of *Strept. hemolyticus* bacteremia. No history of heart or brain disturbances

TABLE II

Solitary Forms in Cases of Rhabdomyoma of the Heart

Author	Heart	Brain	Other Organs	Age	Year	Remarks
1. Hlava [20]	Tumor in left ventricle	Not mentioned	Negative	14 days	1886	Sudden death
2. Wolbach [1]	Tumor in right ventricle arising from septum and posterior median papillary muscle	No tuberous sclerosis	Spina bifida, multiple neuroglia nests in cortex of brain and spinal meninges	10 mos. (female)	1907	
3. Ehrnrooth [45]	Large nodule comprising almost entire left ventricle wall	No gross changes. (No microscopic examination)	Grossly no changes in other organs	7 mos.	1911	Well nourished. Died suddenly after coughing
4. Hisinger-Jägerskiöld [46]	Nodule at apex, size of hen's egg. Comprises greatest portion of the posterior ventricle wall and septum	Not mentioned	Not mentioned	7½ mos.	1916	
5. Amersbach and Handorn [35]	Encapsulated tumor at atrioventricular border, forming a part of the anterior wall of left ventricle, reaching into the septum	No tuberous sclerosis	Negative	7 days (male)	1921	Full term, cyanotic at birth. Died with signs of heart failing slowly
6. Steinbiss [13]	Nodule size of thumb-nail at apex of left ventricle. Heart atrophic	Tuberous sclerosis. Solitary nodules in temporal lobes. Calcified nodules in ependyma	Numerous solitary cysts. Tumors in renal cortex	35 yrs.	1923	Cachectic epileptic
7. Omodei-Zorini [47]	2 cm. tumor in right ventricle wall near apex	No details	No details	2½ yrs.	1923	
8. Sikl [48]	No details available	No details	Hare lip. Cleft palate	9 wks. (male)	1925	Clinical diagnosis of valvular disease

TABLE III

Unclassified Forms in Cases of Rhabdomyoma of the Heart

Author	Heart	Brain	Other Organs	Age	Year	Remarks
1. Schmincke [2]	Diffuse nodules entire myocardium. Right myocardium 1.3 cm. Left myocardium 1 cm.	Negative	Negative	Newborn	1922	
2. Askanazy [10]	No details	No details mentioned in discussion				
3. Lucke [12]	No details	No details mentioned by Goldstein				
4. Steinbiss [13] (Case 7)	No details	No details mentioned			1923	
5. Hieronymi and Kukla [14]	Multiple nodules	Negative	Negative	6 wks. (female pig)	1921	Well developed
6. Billard [15]	Multiple nodules. (No microscopic)	Not mentioned		3 days	1828	
7. Taruffi [22]	No details					

The conclusion that the cells of this tumor probably arise from Purkinje fibers was drawn by many authors (Knox and Schorer,[23] Kawamura,[28] Schulgin,[31] Mönckeberg,[26] Abricossoff,[32] Uehlinger,[33] and Berger and Vallée[30]). Retzer and Aschoff,[34] although not reporting cases, gave support to this view. This theory sounds logical at first glance. The vacuolated appearance, the differentiation into fibrils at the periphery, the restriction of the cross-striations to these fibrils and the richness in glycogen content of the Purkinje cells, all point to similarity to the tumor cells. Tawara found in the ventricular muscle occasional fibrils which are similar to the terminal processes of the conduction system and Rehder found vacuolated Purkinje fibrils in the myocardium of a 46 year old man and a 13 year old boy. These are similar but smaller than the rhabdomyoma cells. Rehder includes an illustration of the 46 year old case in his paper and argues that the location of the tumor in the reported cases, in the anterior ventricular wall, septum, trabeculae and papillary muscles, situated mostly subendocardially, plus the similarity to Purkinje fibrils, permit the conclusion that the tumor arises from the conduction system. Steinbiss, however, points to the fact that the tumors are often found where the conduction system cannot be demonstrated, and further, that if this tumor were a malformation of the conduction system, a disturbance of conduction during life, especially in marked cases, could be expected. But this finding has never been noted. Amersbach and Handorn [35] proved to their satisfaction that there is no connection between the conduction system and the tumor by tracing out both structures in serial section. Rehder believes that the tumor arises from a primitive layer (Mutterboden) common both to the Purkinje fibrils and the myocardial cells before the differentiation into the two muscle systems takes place. On the basis of the available evidence, this view appears to be the most logical explanation.

The true status of the tumor was not settled for a time. Kolisko first stated that the cells are embryonic in type. Cesaris-Demel compared the tumor spaces to spaces he found between anastomosing cells in the hearts of the human embryos. Later, Seiffert, on the basis of the work of Felix [36] on striated muscle in the embryo, studied the embryonic muscle cells of frogs, cats and the human fetus and so compared the tumor cells to embryonic heart muscle cells during the stage of fibril formation, when the fibrils are limited to the periphery

of the cell, while the central portion is filled with a homogeneous substance which does not stain. The part of the sarkoplasm which surrounds the tumor cell remains undifferentiated. Knox and Schorer laid stress upon the clear spaces in the embryonal cells and in modified muscle cells found in adult hearts. Wolbach mentions that he saw similar cells in the moderator band from an infant's heart. In a number of autopsies Rehder noted as an incidental finding small vacuolated areas in the myocardium, which he compared to Seiffert's description of the heart muscle of a 2 month's old human embryo.

Further studies by means of staining reactions established fully the true nature of the tumor. Wolbach, in 1907, using the phosphotungstic acid hematoxylin, and anilin blue stains, was able to demonstrate in the more differentiated portions of his tumor the alternating thick and delicate striations, the sarcous elements and the membranes of Krause in normal muscle, the former staining red with the connective tissue stain, and the latter blue. In the same paper he describes in the large cells with abundant protoplasm, clusters of deeply staining granules without any definite arrangement or connection with one another. These granules are sometimes in pairs, biscuit-shaped, with the flat sides apposed, giving an appearance strongly suggestive of centrosomes. The granules, when stained with the aniline blue stain, are colored a brilliant red with the acid fuchsin. Occasional granules in groups of two to many are connected by delicate fibrils in a more or less orderly fashion. Wolbach concludes that "this is evidently the beginning of muscle fibril formation." This was the first observation of this kind, and many years later (1928) on the basis of further study of this tumor, and a malignant rhabdomyoma of skeletal muscle, Wolbach [37] was able, by reconstructing certain sequences, to show the rôle of the centrosome in myofibril formation. In his later paper Wolbach describes the apparent sequence as follows: "Multiplication of centrioles and centriole clusters; dispersion of centriole clusters, dispersion of centrioles, probably continued division of centrioles and fibrils." Based upon staining reactions and apparent morphological sequences, Wolbach gives the following interpretation: "The granules of centriole origin give rise to the dark disc or 'Q' band; the fibrillary material contracts into the 'Z' band (Krause's membrane)." In addition to clarifying the origin of this tumor, Wolbach's observa-

tions have also given a means of diagnosis of tumors of striated muscle when differentiation is low and cross-striations are not present.

The Cohnheim-Ribbert theory of embryonic rests, activated in response to mechanical disturbances, was early introduced as an explanation of the occurrence of the rhabdomyoma. Seiffert stresses the great opportunity for tissue injury, though not gross in character, in an organ with a development so complicated as that of the heart. Steinbiss takes issue with Seiffert's stand, pointing out that the very rarity of the tumor is the greatest evidence against such a hypothesis. Ribbert [38] later states that he does not believe the vacuolated areas he found strewn through the myocardium of his case are rests of embryonal musculature, but that they are independent shoots (sprouts). The areas he describes are always surrounded by connective tissue and have a larger circumference and thicker striations than in the embryo.

Rehder believes the rhabdomyoma is a tissue malformation and not a true tumor. He calls it a hamartoma (Albrecht [39]) and not a hamartoblastoma, and so would not classify it with true muscle tumors such as rhabdomyoma of the esophagus and bladder. Schmincke [2] also regards the rhabdomyoma of the heart as a malformation, *i. e.*, a hamartoma. He describes it as the end of a continuous chain, at the other end of which are the smallest areas of embryonal muscle tissue in the heart of a newborn. Steinbiss also takes issue against the classification of this tumor as a true neoplasm. He points out that the form of the embryonal cell is retained but the size is greatly increased. There is a retardation in the stage of development, but hypertrophy of the individual elements. With this hypertrophy the cells reach a degree of tissue maturity and proportional to this maturity they lose their potentiality for growth beyond a certain point. This differentiates them from the embryonal rests (Ribbert), which have much greater prospective potentiality, and so metastases and rapid growth are unlikely. No true progressive changes have been described. Occasional mitotic figures, as noted by Bundschuh and Abricossoff can be compared to a similar multiplication in normal striated muscle. The later fate of the tumor also argues against a true neoplasm. Steinbiss describes these changes as regressive in character with nuclear changes, the process becoming plump, the cytoplasm granular and the vacuoles smaller.

Gradually the mass decreases in size and is replaced by connective tissue, to be noted eventually as scars of unknown origin in the myocardium. In one case Rehder reports calcification in a portion of his tumor. He concludes that rhabdomyoma of the heart is a simple embryonal tissue malformation, whose independent growth and complete development have been reached in embryonic time.

Steinbiss denies the necessity of looking for local disturbance factors to explain these tumors. In the presence of their marked association with brain, kidney and skin processes, and various congenital malformations, Steinbiss believes the underlying process must take place early before the differentiation of the germinal layers. He offers a simple, somewhat naïve explanation, which nevertheless has something appealing in its meaning. He regards the whole picture as a surplus formation — a "too much" in the heart anlage, a "too much" in the brain anlage, kidney anlage, etc.

The evidence seems to point against a true neoplastic nature of this growth. At any rate, it appears unsatisfactory to call upon local mechanical disturbances to explain its origin. To put it into the hamartoma grouping and to leave its origin cloaked in the obscurity characteristic of so many deviations from the normal in growth, seems to dispose best of all this perplexing question in the rhabdomyoma literature.

Schmincke's case [2] deserves special mention, since it was the only one of its type in this series. In a newborn infant, he found a heart weighing 46 gm., with measurements of 1.3 cm. and 1 cm. for the right and left ventricular walls respectively. The entire myocardium consisted of rhabdomyoma cells. No mature myocardial cells were found. In the hypertrophied papillary muscles, differentiated cells were present. Uehlinger's case presented certain similar features but contained definite nodules as well. In 1896 Virchow [40] expressed the theory that "idiopathic" hypertrophy of the heart could be explained by a diffuse rhabdomyoma of the myocardium but he lacked a case to demonstrate this. Schmincke, in his report, resurrected the old Virchow theory by illustrating it with his case, and came to the conclusion that diffuse rhabdomyoma of the heart explained the "idiopathic" hypertrophy. We have been able to study several hearts in cases of "idiopathic" hypertrophy and in no case could we demonstrate the presence of rhabdomyoma.

Only a brief discussion of the frequently associated cerebral tu-

berous sclerosis will be given here. The subject will be treated at length in a later paper from this laboratory. A large literature [41] has grown up on the subject of cerebral tuberous sclerosis in the past thirty years and many more cases of tuberous sclerosis than rhabdomyoma of the heart have been reported.

Tuberous sclerosis in instances of rhabdomyoma of the heart was first noted but not described by von Recklinghausen. Cesaris-Demel reported the association in his case, but did not describe the condition. Ponfick was the first to call attention to the association of rhabdomyoma of the heart and tuberous sclerosis and described a diffuse fibrillary gliosis limited to the gray matter. He believed the two conditions to be congenital and in some way related. Bonome-Cagnetto's two cases [42] were also associated with tuberous sclerosis, and he sought an explanation and possible common factor in the evidence of fetal malnutrition with secondary vascular degeneration. Further studies were made by Jonas, Ribbert, and most of the later writers. An examination of the accompanying tables shows that associated with the multiple type of rhabdomyoma, tuberous sclerosis was present in 24 out of 28 cases where the head was mentioned, and with the single type, 1 out of 4 cases.

Also associated with rhabdomyoma of the heart, but to a lesser degree, have been reported kidney tumors [49] of various types and titles, *e. g.*, angiomyosarcoma, angiolipoma and renal adenoma; cysts of the renal cortex, rests of embryonal renal tissue without glomeruli, and adenoma of the skin. The accompanying tables record the association in each instance. Certain other developmental anomalies such as multiple neuroglia rests in the meninges, congenital malformation of the pancreas,[28] as well as the more frequent structural anomalies such as harelip, have also been reported. However, by far the most constant association has been tuberous sclerosis. All six cases reported by Steinbiss had tuberous sclerosis. One of these cases (5) showed degeneration of the cerebral process, with calcification, widespread ossification and formation of marrow spaces.

NOTE: I wish to thank Dr. G. A. Bennett for the photomicrographs and Dr. O. Gates for the pictures of the brain.

BIBLIOGRAPHY

1. Wolbach, S. B. *J. Med. Res.*, 1907, **16**, 495.
2. Schmincke, A. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1922, **70**, 513.
3. Kantzow and Virchow. *Virchows Arch. f. path. Anat.*, 1866, **35**, 211.
4. Skrzeczka, C. *Virchows Arch. f. path. Anat.*, 1857, **11**, 181.
5. Rieder, R. *Jahrb. d. Hamburg. Staatskrankenanstalten*, 1889, **1**, pt. 2, 61.
6. Justi, F. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1896, **7**, 1: Also Lubarsch, *Ostertag Ergebnisse*, 1897, **2**, 574.
7. Seiffert. *Beitr. z. path. Anat., u. z. allg. Pathol.*, 1900, **27**, 145.
8. Kennard, K. S. *New York State J. Med.*, 1921, **21**, 346.
9. Bradley, E. B., and Maxwell, E. S. *J. A. M. A.*, 1928, **91**, 1352.
10. Askanazy. *Verhandl. d. deutsch. path. Gesellsch.*, 1901, **3**, 64.
11. Seiffert. *Verhandl. d. deutsch. path. Gesellsch.*, 1901, **3**, 64.
12. Goldstein, H. I. *New York M. J.*, 1922, **115**, 97.
13. Steinbiss, W. *Virchows Arch. f. path. Anat.*, 1923, **243**, 22.
14. Hieronymi, E., and Kukla, R. *Virchows Arch. f. path. Anat.*, 1921, **232**, 459.
15. Billard. Die Krankhaften Geschwulste, Virchow, **2**, 99.
16. Kolisko. *Wien Med. Jahrb.*, Neue Folge, 1887, **2**, 135.
17. Jonas, W. *Frankfurt. Ztschr. f. Path.*, 1912, **11**, 105.
18. von Recklinghausen. *Monatschr. f. Geburtskunde*, 1862, **20**, 1.
19. Virchow, R. *Virchows Arch. f. path. Anat.*, 1864, **30**, 468.
20. Hlava. *Sborn. lék.*, 1886, **1**.
21. Cesaris-Demel, A. *Arch. per le sc. med.*, 1895, **19**, 140.
22. Ponfick. *Verhandl. d. deutsch. path. Gesellsch.*, 1901, **4**, 226.
23. Knox, J. H. M., and Schorer, E. H. *Arch. Pediat.*, 1906, **23**, 561.
24. Marchand. *Verhandl. d. deutsch. path. Gesellsch.*, 1901, **3**, 64.
25. Rehder, H. *Virchows Arch. f. path. Anat.*, 1914, **217**, 174.
26. Mönckeberg. *München. med. Wchnschr.*, 1914, **61**, pt. 2, 2108.
27. Bundschuh, E. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1912, **54**, 278.
28. Kawamura, R. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1913, **24**, 801.
29. Mittasch. *München. med. Wchnschr.*, 1922, **69**, 571.
30. Walbaum, O. *Virchows Arch. f. path. Anat.*, 1899, **158**, 170.
31. Schulgin, M. *Zentralbl. f. Herz- u. Gefässkr.*, 1913, **5**, 33.
32. Abricossoff, A. J. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1909, **45**, 376.
33. Uehlinger, E. *Virchows Arch. f. path. Anat.*, 1925, **258**, 719.
34. Aschoff. *Verhandl. d. deutsch. path. Gesellsch.*, 1910, **14**, 3.
35. Amersbach and Handorn. *Frankfurt. Ztschr. f. Path.*, 1921, **25**, 124.
36. Felix. *Ztschr. f. wissensch. Zoöl.*, 1889.
37. Wolbach, S. B. *Anat. Record*, 1928, **37**, 225.
38. Ribbert, H. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1915, **26**, 241.
39. Albrecht, E. *Verhandl. d. deutsch. path. Gesellsch.*, 1904, **8**, 89.
40. Virchow, R. *Berl. klin. Wchnschr.*, 1896, **33**, 679.
41. Fischer, W. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1911, **50**, 235.
42. Bonome-Cagnetto. *Cf.* Ref. 1 (Wolbach), also *Atti del Reale Instituto Veneto Scienze Lettere ed Arti*, **43**, pt. 2.
43. Rothe. *Allg. med. centr. Ztg.*, Berlin, 1901, **70**, 175.
44. Kaufmann, E. Lehrbuch der speziellen pathologischen Anatomie, Berlin and Leipzig, 1922.
45. Ehrnrooth, E. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1911, **51**, 262.

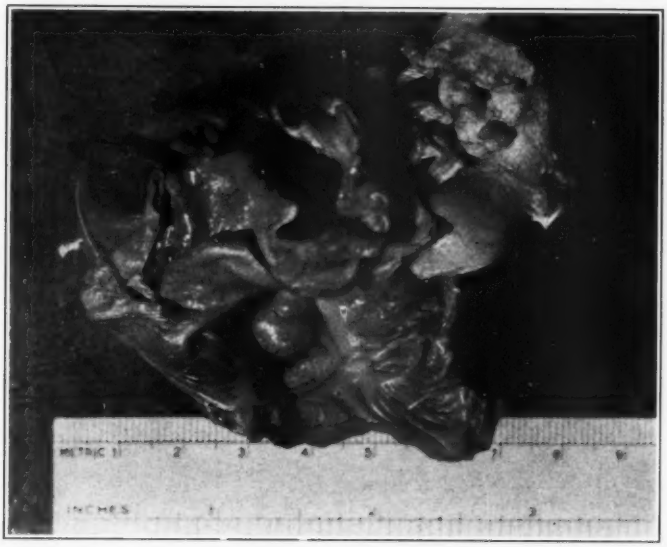
46. Hisinger-Jägerskiöld, E. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1916, **27**, 508.
47. Omodei-Zorini, A. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1923, **34**, 350.
48. Sikl, H. *Čas. lék. česk.*, 1925, **64**, 757.
49. Kirpicznik, J. *Virchows Arch. f. path. Anat.*, 1910, **202**, 358.
50. Berger, L., and Vallée, A. *Ann. d'anat. Path.*, 1930, **7**, 798.
51. Riedmatten. *Travaux Institut Pathologique, Lausanne*, 1904, **3**, 167.
52. Taruffi. *Compendio di anat. patol.*, Bologna, p. 311.

DESCRIPTION OF PLATES

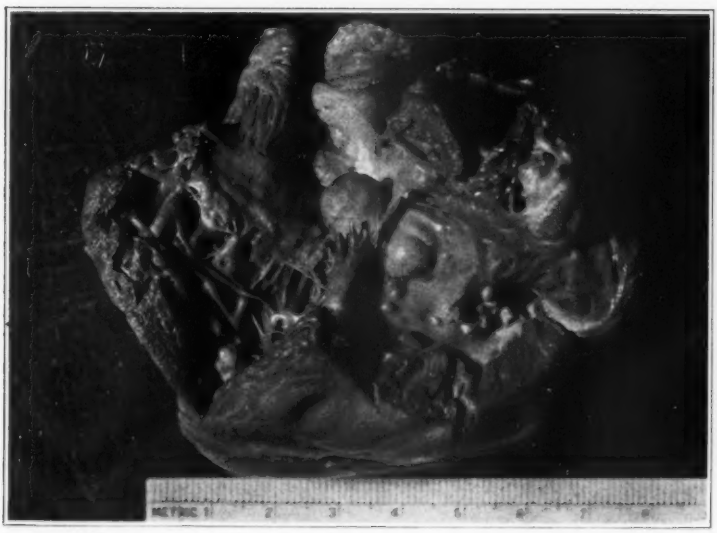
PLATE 23

FIG. 1. Heart with right auricle exposed. Large tumor mass obstructs tricuspid valve orifice.

FIG. 2. Heart with right auricle and right ventricle exposed. Note extension of large tumor mass from auricle into ventricle. Many small tumor nodules can be seen on posterior wall of right ventricle.



1



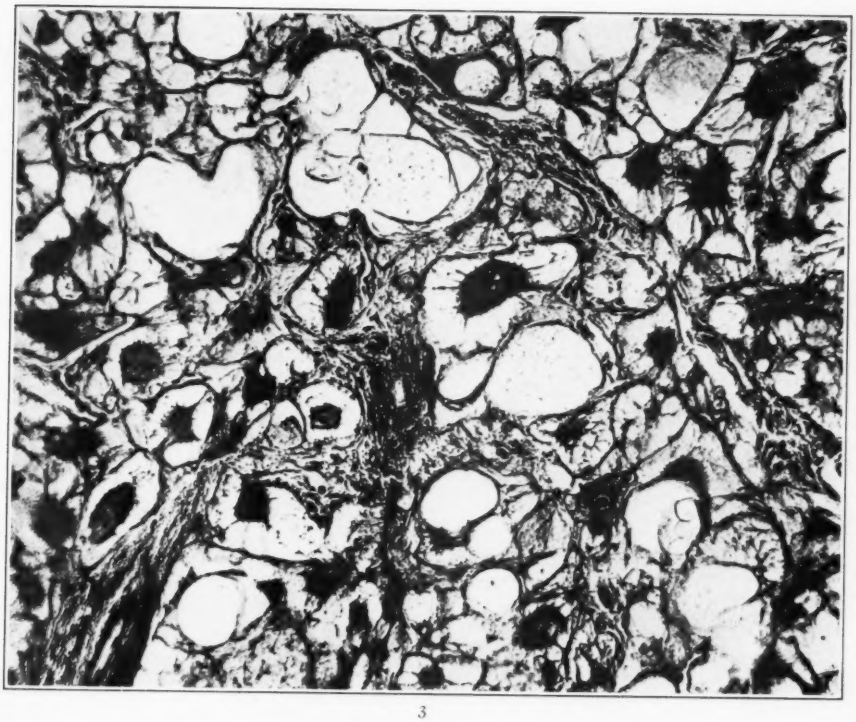
2

Farber Congenital Rhabdomyoma of Heart

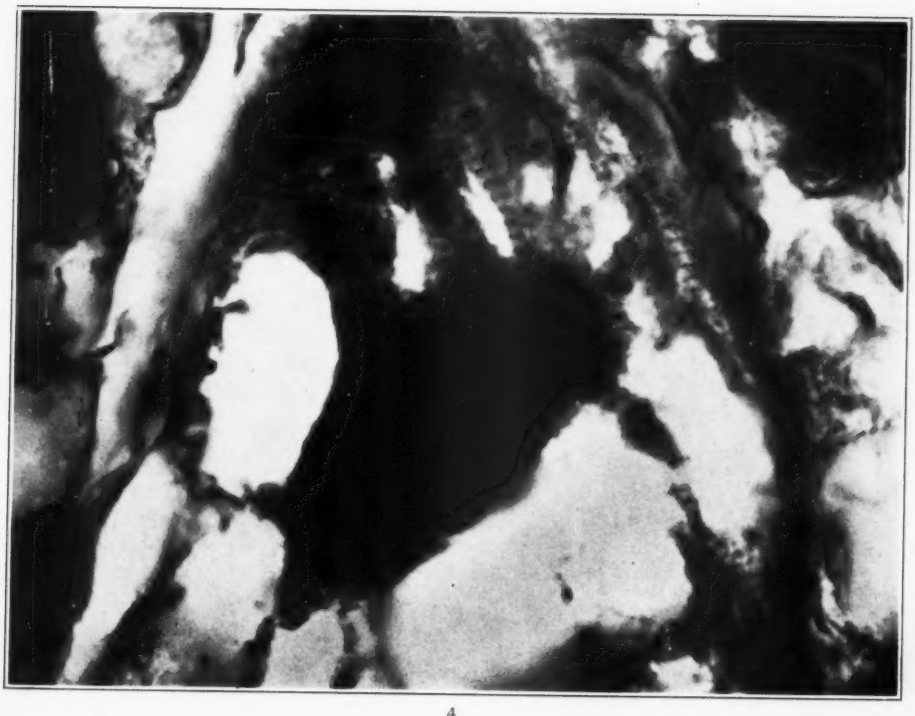
PLATE 24

FIG. 3. Photomicrograph of tumor nodule. Phosphotungstic acid hematoxylin stain. Typical view of architecture of tumor. Note "spider" cells and large spaces. × 200.

FIG. 4. Photomicrograph of tumor. Phosphotungstic acid hematoxylin. Shows large cell with cross-striations in fibrils and at periphery. × 1600.



3



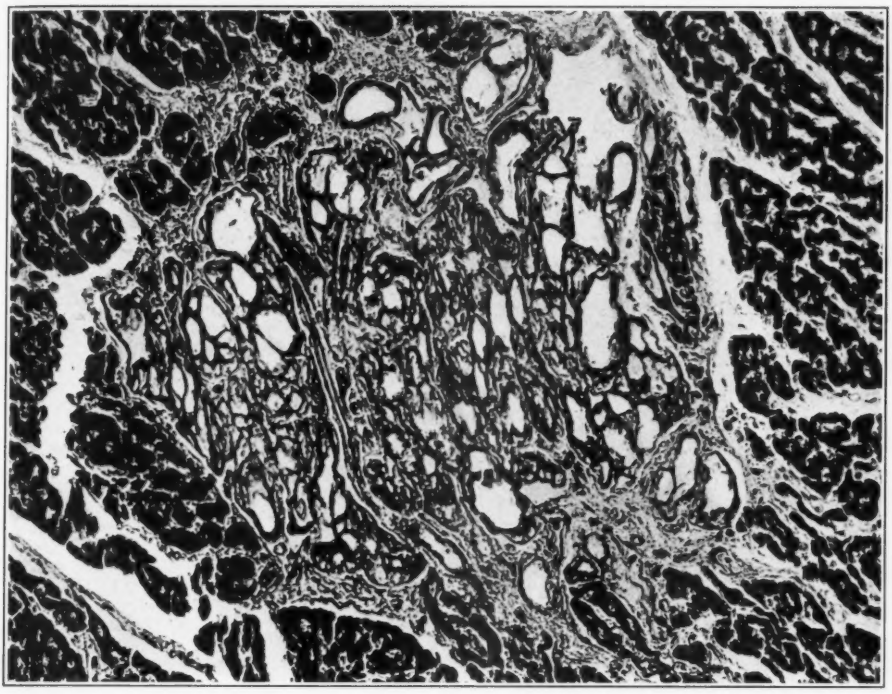
4

Farber Congenital Rhabdomyoma of Heart

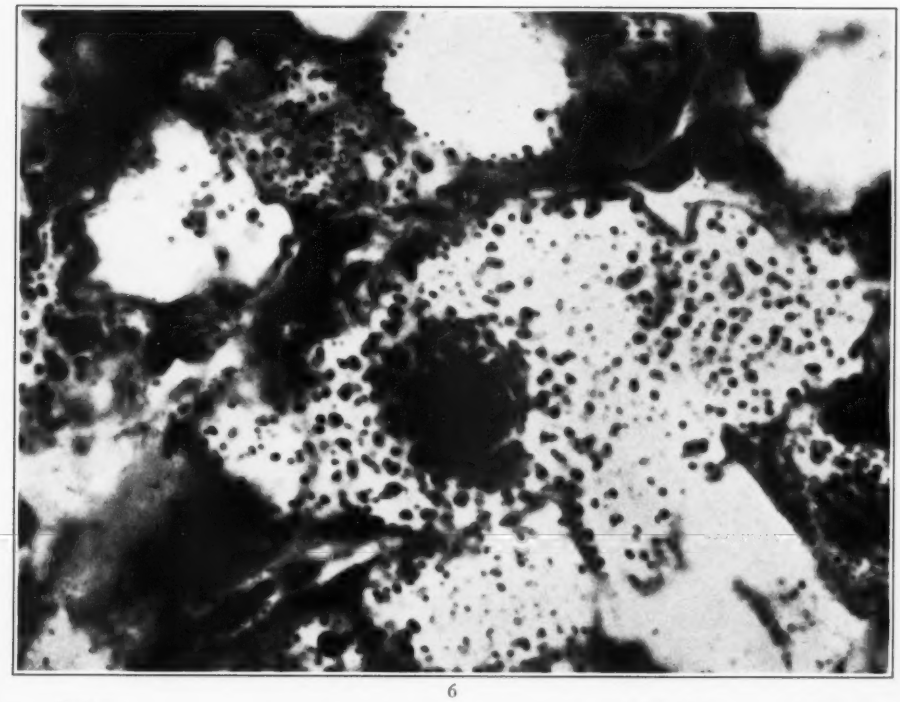
PLATE 25

FIG. 5. Photomicrograph from grossly normal myocardium. Phosphotungstic acid hematoxylin stain. Shows isolated collection of tumor cells with empty spaces. × 200.

FIG. 6. Photomicrograph from tumor nodule. Best's carmine stain. Note large cell with large numbers of glycogen droplets. × 850.



5



6

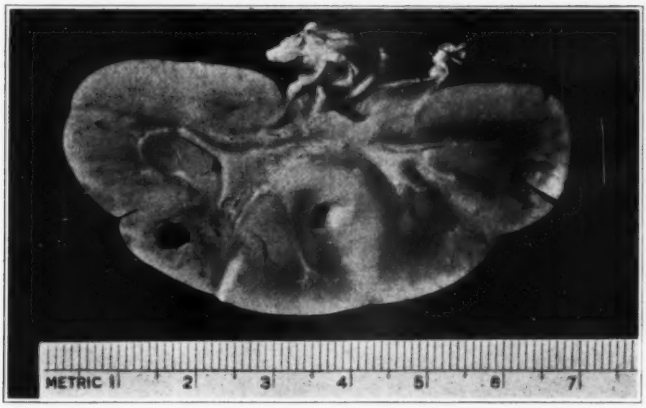
Farber Congenital Rhabdomyoma of Heart

PLATE 26

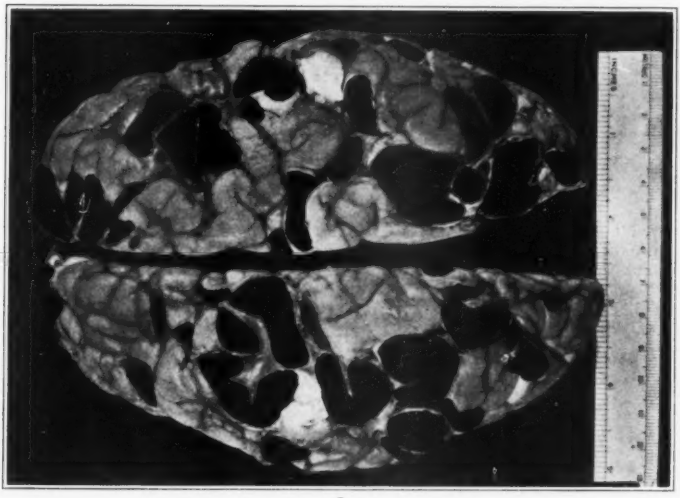
FIG. 7. Kidney with several cysts.

FIG. 8. Brain showing convexity. Shaded areas represent distribution of the tuberous sclerosis.

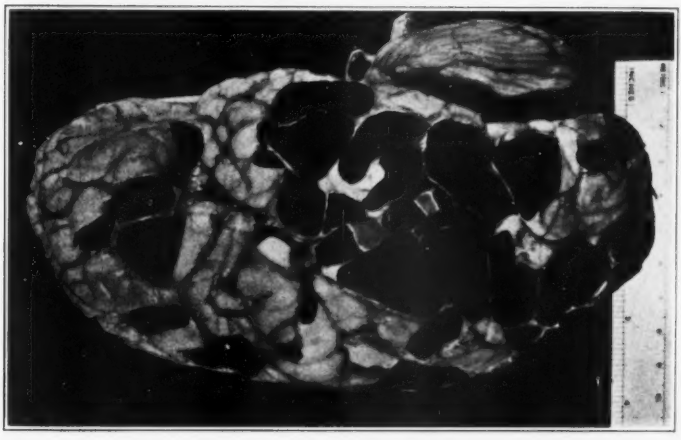
FIG. 9. Brain, lateral view, showing distribution of tuberous sclerosis.



7



8



9

SUPRAVITAL STAINING WITH SILVER AMMONIUM CARBONATE *

F. A. McJunkin, M.D.

(From the Department of Pathology, Loyola University School of Medicine, Chicago, Ill.)

Since impregnation with silver makes visible in a remarkable way structures otherwise not well seen, it has obvious value. However, scarcely any other substance colors such a wide variety of related and unrelated cellular products as does silver. In the connective tissue group especially fine shades of distinction have been attempted with the silver impregnations and many conflicting views have consequently arisen. The application of the various methods has been almost exclusively to dead tissue, and with few exceptions to dead fixed tissue. The outstanding exception is the method of Taft and Ludlum,[1] in which they treated unfixed brain with argyrol. In their preliminary report they state that they used fresh tissue from experimental animals. The first experiments made in the present investigation were with argyrol which was injected directly into living tissues by a method previously used to obtain supravital staining with neutral red.[2] The phagocytes were found to react as they do to carbon in fine suspension [3] and not as they do to neutral red. Silver ammonium carbonate was substituted for the argyrol and found to penetrate the cytoplasm of certain living cells as readily as the dyes used in the supravital stains. Once within the cells the silver was reduced and precipitated in the usual way, where it remained permanently. The chief handicap of supravital staining with neutral red has been inability to retain the dye without change in sections of the embedded tissue. The author[2] published the method for mordanting the neutral red with chrome salts. Later several methods using chrome salts as a mordant were published by others, but all were of limited value on account of solution of much of the dye during the embedding and staining processes. The method had some value since a little of the dye was retained in the sections of tissue and some of the cellular relationships could be distinguished.

* Received for publication December 31, 1930.

Technical Methods

The directions given by Foot [4] were followed in the preparation of the silver ammonium carbonate. Full-grown rats were etherized and bled from the heart. About 10 cc. of the silver solution were injected into the portal vein to free the liver of excess blood and finally the solution was injected directly into the liver until it lost color and assumed a translucent appearance. The solution was injected directly into the substance of the spleen until it was distended to twice its normal thickness and until the tissue became white at points of greatest concentration. The lymph nodes situated near the aortic bifurcation were distended with the solution. After 20 to 30 minutes, the spleen, lymph nodes and liver were fixed in 10 per cent formalin. Paraffin sections prepared in the usual way were stained with hematoxylin and eosin. The reduced silver is not affected by the usual staining methods, but any excess of dye obscures the yellowish silver granules. The best results have been obtained by staining lightly, about 10 to 15 seconds in Harris' hematoxylin without acetic acid, and in dilute eosin about 10 seconds. As in the use of neutral red it was found that the silver solution kills the cells at once where it reaches them in greatest concentration, but that in adjoining territory where its strength is reduced by contact with the dead tissue and by diffusion the supravital effect is produced. In addition to the full strength solution all dilutions, 1:2, 1:3 and so on to 1:10, have been injected into the organs of numerous rats. Dilutions above 1:4 are usually ineffective. A 1:2 dilution with distilled water, or the undiluted solution is the best for all tissues. In the dead tissue areas the silver staining is diffuse and has extended to nuclei and intercellular substance. The yellow color of the nuclei becomes obscure after application of the hematoxylin. Such areas must be avoided in a study of the vital reactions.

Histological Findings

Spleen: Distention of the organ by the silver solution and its fixation in the distended state makes conspicuous the structural relations described by Robinson.[5] The solution does not penetrate the malpighian corpuscles well, and attention has been directed especially to the venous side of the circulation. The veins leave the capsule and trabeculae, lose their connective tissue support and pass

into the lobules as capillaries consisting of a single layer of endothelium. Often the terminus of the capillary can be distinguished. Usually the lumen widens and the narrow endothelium flares out into a network of large reticular cells. The size of the lumen depends on its distention by the injected fluid and not infrequently the two rows of endothelial cells terminate with the network of silver-staining cells beyond. The terminal expansions may be lined on one side with flat cells and on the other with reticular cells (Fig. 4). Near the splenic capsule the injection is best. The histiocytes are made conspicuous by the yellow granulation. Without the silver the cytoplasm of these cells stains lightly and the cell outline is indistinct. Where the histiocytes are spread apart they are seen to be connected by innumerable fine fibrils which do not react to the supravital silver but which take the stain in the areas of dead tissue. The silver granules are irregular in shape, size and distribution. In most of the cells the granules are scattered throughout, but in some there is a mass of granules occupying one segment of the cell. The nuclei of the histiocytes are relatively small and have some tendency to an oval shape. The flat endothelial cells contain no silver. In most of the capillaries there is not much evidence of the flat cells undergoing a gradual transition to form the larger histiocytes.

Liver: The injection of the liver is not as satisfactory as that of the spleen. The sinusoidal endothelium is pressed against the hepatic cells. Also, so much of the silver is absorbed by the abundant parenchyma that it is difficult to regulate the strength of the solution. However, in selected areas the Kupffer cells are distinct and in their cytoplasm is seen a variable number of irregular silver granules, unevenly distributed. There is little evidence of any massing of the granules about a centrosphere. The largest of this type of cells shows a delicate cytoplasm resembling the histiocytes of the spleen, but the network of fibrils about them and connecting them is not shown. This appears to be dependent on a stretching and spreading of the vascular network which does not follow the injections as it does in the spleen.

Lymph Nodes: In general structure and in function the reticular tissue of lymph nodes is unlike that of the spleen. Both these organs ingest particulate matter and segregate colloids, but in different fashion. In the medulla the cells of the sinuses are well separated by the force of the silver injection. Many of the cells reveal graceful

cytoplasmic processes, often two in number, that connect them (Fig. 5). These cells are much like the phagocytes that grow in tissue cultures of lymph nodes.[6] Free in the sinuses, there are many large round cells that have separated after the injection and before fixation. In both attached and detached cells the cytoplasm contains large numbers of silver granules, regular in size and shape. In most cells the granules are scattered throughout the cytoplasm, but in some they are clumped in one mass. The silver preparations show to be true what was little more than surmised in the tissues treated with neutral red. The reacting cells are not confined to the sinuses but extend into the dense follicles where groups of non-reacting lymphocytes lie in their interstices. In this dense tissue the silver granules are usually in a single group and not infrequently a clear space appears at the center of the mass. The silver-reacting cells in the sinuses connect with those in the follicles. Both are very different in appearance from the histiocytes of the spleen. The cytoplasm is dense and the cell outline perfectly distinct. The silver granules are spherical and of uniform size. The cells readily round up and separate. The nuclei are relatively large and not infrequently cupped at one side (Figs. 2 and 3). The narrow flat cells of the sinus walls show no reaction in their scant cytoplasm.

Discussion

Any investigation of free cells should take into account their origin from a fixed tissue. A prevalent view in regard to free mononuclear phagocytes is that they do not arise from a fixed tissue but that they exist instead as a distinctive cell type that separates during embryonic development to persist in various organs and tissues throughout life as wandering or wandering "resting" cells. Maximow [7] was of the opinion that one fraction of the mononuclear phagocytes was of this type and that the other was derived from lymphocytes by a phagocytic transformation. Certainly most free cellular elements such as lymphocytes, granular leukocytes, red blood corpuscles and blood platelets are fixed-tissue derivatives and the author [3] has for many years adhered to the view that the mononuclear phagocytes of the higher vertebrates have a base of supply in the fixed tissues.

The observations made on the supravital silver stains strengthen the view previously expressed by the author,[8, 9] which gave recogni-

tion to essential differences between the so-called reticular cells of lymphoid tissue and reticular cells of sinusoidal organs such as the spleen and liver. Although the functions performed by these two groups are so very different they are quite generally classed together as the reticulo-endothelial system. Sabin, Doan and Cunningham [10] in the examination of splenic puncture material in the supravital films found a rosette type and a diffuse-granule type of cell. Without an investigation of the *in vivo* relationship of the reacting cells they concluded that the rosette form was derived from reticular tissue in the spleen and other sinusoidal organs but not from the lymph nodes, since they found few of the rosette forms in the lymph gland punctures. The diffuse-granule cell was thought by them to be derived from capillary endothelium. The evidence now is that the arrangement of supravital granules within the mononuclear phagocyte depends on the age and functional activity of the cell rather than on its kind.

Histiocytic Tissue: The nomenclature used in connection with the mononuclear phagocytes is burdensome, and it is desirable not to make it more so. Fortunately, the mononuclear cells present in the peripheral blood are almost universally called monocytes. Tissue from which they arise may well be called monocytic tissue. To exclude a misunderstanding, histiocytic tissue is, in this article, applied to the sinusoidal endothelial network with the implication that some cells may separate from it to become free histiocytes. The term has, of course, been used in a much broader sense. The view that the vascular network may, during embryonic development, arise *in situ* from the mesenchyme is well supported, but there is less evidence that endothelium may differentiate from a connective tissue base in the fully matured organism. There are two main explanations for the characteristic vascular network of sinusoidal organs. Maximow maintained that it was unrelated to endothelium and called the cells composing it histiocytes. This implies that in several organs the blood passes from its endothelial-lined channels to enter spaces lined by an unrelated type of cell. The alternative view is that endothelium as a mesenchymal derivative may occur in either of two forms. The difference between ordinary flat endothelium and reticular cells is not necessarily interpreted as due to a difference in cell type. In granulation tissue experimentally produced beneath skin surfaces the author [8] found that the capillary endothelium of the

usual non-phagocytic type became large, phagocytic, and even assumed a branching reticular appearance when it was sufficiently stimulated (Fig. 1). Again, in observations on granulation tissue in the liver,[11] it was seen that the sinusoidal endothelium, normally phagocytic in this location, became inactive and non-phagocytic when contact with hepatic parenchyma was lost. In the spleen the structural relations indicate an identity of endothelium and reticular cells. The two are continuous and the histiocytes in part form the walls of the terminal capillaries. It is likely that the two are interchangeable in form and function. When the cell shrinks it becomes non-phagocytic and non-reactive to solutions of silver. Under normal conditions, the terminal or functional cells are phagocytic for coarse particulate matter such as senile or injured red blood corpuscles and fragmented lymphocytes. In the performance of their function of ridding the blood of undesirable particulate matter the histiocytes tend to remain anchored by the numerous fine fibrils that connect them to form the splenic pulp. They do not have a great tendency to round up and float free for mobilization at a distance. Such behavior indicates that their activity is largely local. The capacity of these cells to incorporate and segregate the silver salt is related to their selective activity for colloids. The argyrophilic sinusoidal endothelia or histiocytes made up of the terminal cells of the vascular system are, therefore, more than mere tubes for blood transportation. It is the exercise of these added functions that is responsible for the change from inactive flat cell to phagocytic histiocyte. The difference between flat endothelium and histiocyte appears less than the usual difference between duct epithelium and the secreting portions of glandular organs. The relationship, however, is somewhat analogous.

Monocytic Tissue: Evidence of the origin of monocytes from lymphoid tissue is overwhelming. Maximow[7] maintained that these cells constitute a lymphocyte-monocyte group with the lymphocytes on stimulation readily becoming monocytes. That the two may arise from a single stem cell is suggested by the extension of the argyrophilic cells from the sinuses into the follicles as described in connection with the lymph nodes. These solid cords of reacting monocytic cells may to some extent represent potential lymph channels, but it must be kept in mind that their proximity to the medulla may be explained by the successful penetration of this tissue by the

injected fluid. Naturally, the silver does not penetrate the solid tissue readily, and as a result the entire distribution of the monocytic tissue has not been determined. The fixed monocytic cells outside the sinuses are rounded, and within the sinuses, elongated. In the former the argyrophilic granules are often grouped into a single mass, while the cells within the sinuses usually show more than one group of granules, or frequently the cells present a diffuse granulation. Great numbers of the cells round up and separate after the injections are made. These free cells have all the characters of the blood monocytes. The monocytic tissue plays a dominant rôle in diseases such as typhoid fever and exudative tuberculosis. It is readily stimulated to form large epithelioid cells with great masses of supravital granules and to participate in the formation of foreign body giant cells. As a circulating leukocyte, the monocyte appears in inflammatory foci almost as quickly as does the granular leukocyte. Although it may undergo mitosis in the lesions, it is recruited chiefly from the lymphoid tissue where the quantity of monocytic tissue almost equals that of the lymphocytic.

Conclusions

1. Silver ammonium carbonate may be used to mark living cells supravitally.

2. By supravital staining with silver ammonium carbonate the origin of the monocytes is seen to be from the silver-marked portion of lymphoid tissue.

3. The histiocytic tissue of the sinusoidal organs also reacts to supravital silver but the response is unlike that of the monocytes.

4. The two component parts of the so-called reticulo-endothelial system are so unlike in function and structure that they should not be grouped together. The hystiocytic tissue of the sinusoidal organs consisting mostly of anchored cells functions chiefly as a fixed tissue. On the other hand, the monocytes originating in the lymphoid tissue are a normal element of the circulating blood and may be concentrated quickly in any tissue or cavity.

REFERENCES

1. Taft, A. E., and Ludlum, S. de W. *Proc. Soc. Exper. Biol. & Med.*, 1930, **27**, 582.
2. McJunkin, F. A. *Am. J. Path.*, 1925, **1**, 305.
3. McJunkin, F. A. *Am. J. Anat.*, 1919, **25**, 27.
4. Foot, N. C. *Am. J. Path.*, 1928, **4**, 525.
5. Robinson, W. L. *Am. J. Path.*, 1930, **6**, 19.
6. McJunkin, F. A. *Arch. f. exper. Zellforsch.*, 1926, **3**, 166.
7. Maximow, A. A. *Physiol. Rev.*, 1924, **4**, 533.
8. McJunkin, F. A. *Am. J. Path.*, 1928, **4**, 587; *Am. J. Path.*, 1930, **6**, 39.
9. McJunkin, F. A. *Proc. Soc. Exper. Biol. & Med.*, 1925–26, **23**, 64; *Arch. Path. & Lab. Med.*, 1926, **2**, 815.
10. Sabin, F. R., Doan, C. A., and Cunningham, R. S. *Contrib. Embryol.*, 1925, **16**, No. 82, 125.
11. McJunkin, F. A. *Am. J. Path.*, 1931, **7**, 9.

DESCRIPTION OF PLATE

PLATE 27

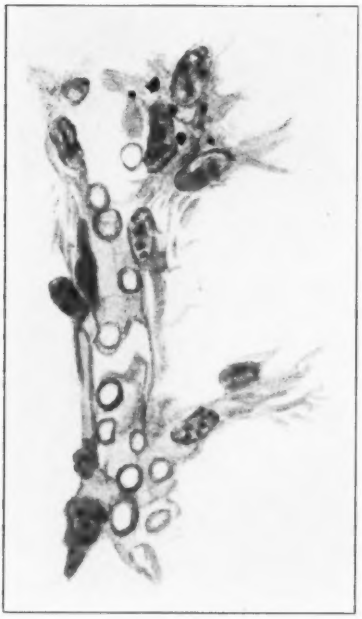
FIG. 1. Capillary in subcutaneous granulation following large injections of trypan blue, and finally, intravenous injection of India ink. The phagocytic cells containing ink particles are structurally like the histiocytes of the spleen.

FIG. 2. Lymph node. Free monocytic cells within sinuses. The two lymphocytes show no silver granulation.

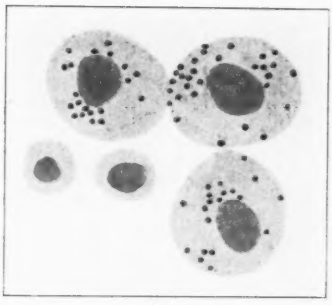
FIG. 3. Lymph node. A small group consisting of two silver-reacting monocytic cells and two lymphocytes outside the sinuses.

FIG. 4. Spleen. Terminal capillary with histiocytic silver-reacting cells on one side.

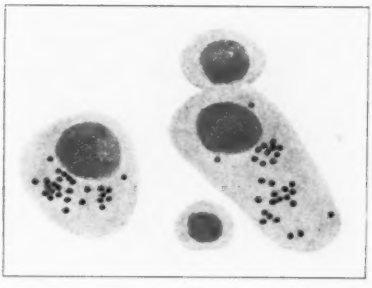
FIG. 5. Lymph node. Monocytic silver-reacting cells of medullary sinuses.



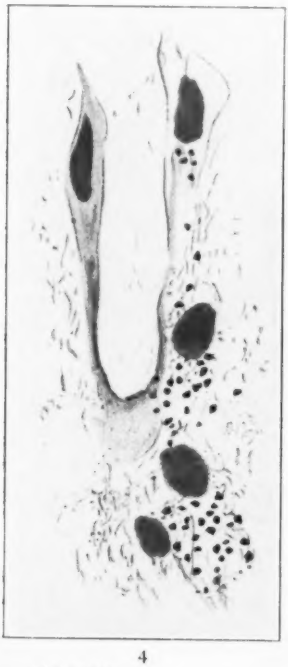
1



2

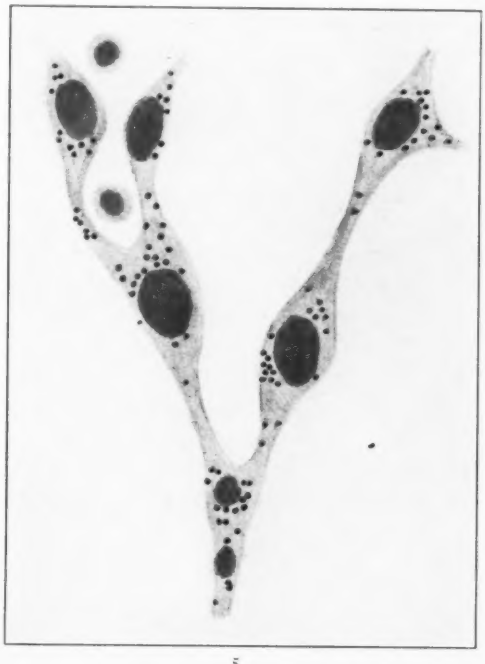


3



4

McJunkin



5

Supravital Staining with Silver

SUSCEPTIBILITY OF THE GOPHER (CITELLUS TRIDECEMLINEATUS) TO MYCOBACTERIUM TUBERCULOSIS *

WILLIAM H. FELDMAN, D.V.M., M.Sc.
(From the Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minn.)

In order to determine the anatomical reaction of the thirteen-lined ground squirrel, *Citellus tridecemlineatus* (Mitchell) to the three bacillary types of *Mycobacterium tuberculosis* the following experiment was performed.

Twelve adult animals were captured in their natural haunts and divided into three lots, each containing four animals. The animals in each lot were inoculated with 0.5 cc. bacterial suspensions prepared from 48-day-old cultures of *Mycobacterium tuberculosis* as follows: two intravenously, one subcutaneously, and one intraperitoneally.

The organisms used were original strains of *Mycobacterium tuberculosis* which had been isolated from human, bovine and avian sources and each had been proved by tests of pathogenicity with guinea pigs, rabbits and chickens to be true to type. After being inoculated, each lot of four animals was placed in a separate cage for observation.

RESULTS

LOT 1: Injection was made with *Mycobacterium tuberculosis* of bovine origin. About six weeks subsequent to the injection two of the animals (Gophers 2 and 3) escaped at feeding time and successfully avoided recapture. One had been inoculated intravenously and the other intraperitoneally. Of the two remaining animals the one which had been inoculated intravenously (Gopher 1) died after thirty-six days. The lungs had been eaten by cage mates, but the abdominal viscera were intact. The spleen was slightly enlarged and contained a few grayish white foci. Microscopic examination disclosed numerous tuberculous lesions throughout the liver and spleen, but lesions were not observed in the kidney. Gopher 4, which had been inoculated subcutaneously, appeared weak after a lapse of forty days, and was killed for autopsy. The animal was in good

* Received for publication December 12, 1930.

flesh and without gross evidence of disease. Microscopic examination disclosed lesions of a tuberculous character in the liver and spleen, but the lungs and kidneys escaped demonstrable involvement.

LOT 2: Injection was made with *Mycobacterium tuberculosis* of avian origin. Gopher 6, which had been given an injection intravenously, died approximately three hours after injection. Gopher 7, which was given an injection intravenously, was killed for autopsy after sixty-six days. Grossly the only lesions were multiple, minute grayish foci in the liver. Many tuberculous lesions were present in the liver and spleen, but in the lungs and kidneys lesions were not found. Cultures were obtained from an emulsified portion of the liver, and a chicken that was inoculated intravenously with 1 cc. of the same emulsion was found to have tuberculous lesions when killed for autopsy sixty-seven days after the inoculation. A guinea pig, when given an injection with a portion of the liver emulsion of Gopher 7, failed to reveal lesions when killed for autopsy after sixty-one days. Gopher 5, which had been inoculated subcutaneously, was killed for autopsy after ninety-one days, and lesions were not found on gross or microscopic examination. Gopher 8, which had been inoculated intraperitoneally, was also killed for autopsy after ninety-one days and was without discernible alterations in either the lungs or the kidneys, but a few definitely tuberculous lesions were present in the liver and spleen.

LOT 3: Injection was made with *Mycobacterium tuberculosis* of human origin. One of the animals which had been inoculated intravenously, Gopher 10, died forty-five days after the injection. Before the animal was found, all the viscera had been eaten by the other animals in the cage, and no suitable material remained for study. Gopher 9, which was inoculated intravenously, died in forty days and numerous and extensive tuberculous lesions were visible grossly throughout the liver, spleen and lungs. A few small tuberculous foci were present in the kidneys. The lesions were most numerous in the medullary portion of the organ. Gopher 12, which had been inoculated subcutaneously, was without demonstrable lesions when killed for autopsy sixty-six days afterward. Gopher 11 died seventy-eight days after receiving an intraperitoneal injection of the bacterial suspension. At autopsy extensive lesions of tuberculosis were observed throughout both lungs (Fig. 1). A few nodular lesions

were also present in the serosa of the intestines. Although the spleen was perhaps slightly enlarged, definite lesions were not seen. Grossly, the liver and kidneys appeared normal. Microscopically there were numerous and extensive lesions of a tuberculous character in the spleen and the liver, but the kidneys were not involved.

Histopathology

From all the tissues obtained for histopathological study two series of sections were prepared. One series was stained with the usual hematoxylin-eosin combination and the other was stained with carbol fuchsin-hematoxylin for the purpose of staining acid-fast bacteria which might be present. Considering first the tissues obtained from the animals in which there were established lesions of tuberculosis following the injection of organisms of bovine origin, the most extensive lesions were found in the tissues of the animal inoculated intravenously. Both the liver and spleen contained many spherical collections of monocytic cells among which were large numbers of acid-fast bacteria. Very few of the monocytes had assumed an epithelioid appearance, although caseation necrosis was beginning in the center of many of the collections. Encapsulation or calcification was not seen. Associated with most of the lesions, particularly the smaller or earlier ones, were characteristic giant cells. These cells were of two general varieties. In one the nuclei were arranged at the periphery of the cytoplasmic mass and in the other the nuclei were grouped more or less centrally. Each of the giant cells possessed large numbers of acid-fast organisms. The lesions which were limited to the liver and spleen failed to show any particular predilection for a specific portion of the respective organs, except it was noted that in the spleen of the animal which had been inoculated subcutaneously, the lesions were in the splenic pulp and not within the splenic corpuscles.

Except for those lesions which occurred in the animal inoculated intravenously, the lesions that resulted as a consequence of the organisms of avian origin were much fewer than those which occurred following the injection of the mammalian strains of *Mycobacterium tuberculosis*. They were, however, essentially of the same character, consisting of collections of monocytic cells with a tendency to undergo caseation necrosis (Fig. 2). The only definite epithelioid

cells observed were those in the splenic lesions of the animal which had been inoculated intravenously (Gopher 7, Fig. 3). The lesions in this instance had their inception within the splenic corpuscles but were limited to one area near the periphery. In no instance were the lesions observed to occupy the central portion of this structure. In the spleen of Gopher 8, which had been inoculated intraperitoneally, the lesions consisted almost entirely of large multinucleated giant cells, often in clusters of three to five. Although these structures were identical in appearance to those definitely associated with true tuberculous lesions, acid-fast bacteria could not be demonstrated within them.

The liver of Gopher 7, which had been inoculated intravenously, contained a large number of focal lesions, and acid-fast organisms were numerous in each. Neither encapsulation nor calcification was observed.

By far the most extensive lesions of tuberculosis occurred in those animals which received *Mycobacterium tuberculosis* of human origin. The extent and character of the lesions, however, appeared to be quite dependent on the route of inoculation, for although the lesions were exceedingly extensive in the animals which had been given injections intravenously, none could be found in the tissues of the one inoculated subcutaneously. The animal that was inoculated intraperitoneally, although possessing a great number of lesions, many of which were extensive, did not have the widespread malignant type of lesions which were so noticeable as a consequence of the intravenous exposure. In the animal inoculated intravenously the lesions of the liver and spleen, which had replaced much of the normal tissue of these organs, were diffuse, and many of the monocytic cells had undergone coagulation necrosis. Epithelioid cells were not apparent. In those lesions in which the cellular integrity remained intact the monocyte of the early tubercle was the cell which was present.

Giant cells, some of which were very large, were exceedingly numerous in the hepatic and splenic lesions where they were associated with the collection of monocytic cells making up the lesion (Fig. 4). Acid-fast bacteria were present in large numbers in all of the lesions of the liver and spleen.

The lungs of the gophers that were inoculated with the organism of human origin intravenously and intraperitoneally were involved

in a most intensive tuberculous process (Fig. 1). Although many tubercles were present, many of which were conglomerate, a cellular reaction indicative of excessive tuberculous infection was apparent throughout the entire substance of the organ. One of the lungs contained numerous large tuberculous abscesses consisting of caseated cellular débris surrounded by a rather wide, fibrous capsule. Enormous numbers of acid-fast bacteria were present, particularly in the peripheral zone of each of the abscesses. Many of the bronchi and bronchioles of the lungs of the animal inoculated intravenously were practically occluded by an exudative substance which contained acid-fast bacteria in large numbers.

Giant cells, which were so numerous in the liver and spleen of the gophers that were inoculated with the organisms of human source, were singularly absent in the lung tissue observed. Likewise calcification was not seen.

Comment

A review of the literature indicates that the common striped gopher has not heretofore been used to any extent in experimental tuberculosis. One reference only was found and this pertained to the use of this animal as a substitute for the guinea pig as a diagnostic aid in obscure tuberculous infections. The report referred to was by Hewetson,[1] a physician located at the time (1905) at Pincher Creek, Alberta, Canada. He found it difficult to obtain guinea pigs, and the prevalence of the gophers in that locality suggested the possibility of using the latter animal in the diagnosis of tuberculosis. He gave each of nine gophers an injection with 2 cc. of a fluid containing tuberculous sputum; in the animals that died thirty to forty days afterward, enlargement of the spleen was noted, together with lesions of a tuberculous character in this organ and in the liver. Lesions were not observed in the lungs. Hewetson concluded that the susceptibility of the gopher to tuberculosis was established and that those living in regions where these animals were numerous might use them in the laboratory diagnosis of tuberculous infections of an obscure character.

Hewetson failed to designate specifically the genus and species of the animals in his experiment, but since the striped gopher is said to be prevalent in that part of Canada in which his observations were made it seems reasonable to assume that some species of Citellus was used.

From the results of my studies it is apparent that there exists a difference in the susceptibility of the striped gopher to the three forms of *Mycobacterium tuberculosis*. The difference in the resistance to each of the three forms of the organism seems to be relative and dependent to some degree on the route of inoculation. In the animals which were given injections intravenously, well defined lesions of tuberculosis developed, regardless of the source of origin of the infecting bacteria. However, the organisms of avian origin proved less successful in inciting lesions when they were introduced subcutaneously and intraperitoneally. Although some of the animals that were injected with the human and bovine forms of the organism died spontaneously within thirty-six to forty days after the injection, those that received organisms of avian origin lived until they were killed for autopsy sixty-six to ninety days after inoculation. This indicates the possession by the gopher of a greater resistance to the avian form of the organism than to either the human or bovine form. The resistance of the gopher to the avian form of *Mycobacterium tuberculosis* was further evident from the fact that even though numerous lesions occurred in the liver of the animal injected intravenously with this organism, the lungs were without demonstrable lesions. This suggests that the lungs of the gopher possess a peculiarly efficient resistance to the avian form of *Mycobacterium tuberculosis* similar to that observed in the lungs of the dog.[2]

The cellular reaction to the organism of tuberculosis in each of the animals was essentially the same, regardless of its source of origin. Any differences noted were largely differences in the extent of the lesions rather than in their fundamental histological character. The unusually large giant cells which were so numerous in association with many of the cellular reactions would seem to be of some significance in the attempt on the part of the tissues to establish and maintain a protective mechanism against the bacteria of tuberculosis. The apparent absence of these cells in the pulmonary lesions, however, is difficult to explain. Perhaps their formation is an index to the degree of resistance exhibited by the monocytic cells of the invaded tissues to the infecting organisms, since the pulmonary lesions in the tissues studied showed very little, if any, arrestment of the disease.

The failure to observe lesions of tuberculosis in Gopher 12, which had been inoculated subcutaneously with the bacterial suspension

of human origin, would make one hesitate to accept the conclusion that the striped gopher might be used as a satisfactory substitute for the guinea pig in determining obscure tuberculous infections. The animal in question received a dose of virulent organisms many times greater than would be likely in material obtained from clinical sources. Yet after a lapse of sixty-six days demonstrable lesions failed to develop. The number of animals used in this experiment constitutes a rather small group from which to draw definite conclusions, but the absence of lesions in Gopher 12 seems of sufficient significance to question the reliability of the use of the gopher as a routine diagnostic aid in determining tuberculous infections. Although infection would probably follow in every instance if sufficient virulent organisms were introduced intravenously, for technical reasons this procedure is not a practical one so far as the gopher is concerned.

Summary and Conclusions

1. With the use of original strains of *Mycobacterium tuberculosis* of human, bovine, and avian origin, a series of gophers (*Citellus tridecemlineatus*) was inoculated to determine the susceptibility of this animal to the respective bacillary forms of the organism of tuberculosis. With each organism two animals were injected intravenously, one subcutaneously, and one intraperitoneally. Two of the twelve animals injected escaped after being under observation for six weeks and were not recaptured, and one animal died three hours subsequent to injection. Of the remaining nine gophers, four died after intervals of from thirty-six to seventy-eight days, and the others were killed for autopsy at from forty to ninety-one days after the injection.

2. Definite lesions of tuberculosis were observed as a consequence of each of the three forms of *Mycobacterium tuberculosis*, with the degree or extent of the infection varying with the route of inoculation, being most pronounced in the animals that were inoculated intravenously. A histopathological study of the lesions in each of the animals was made.

3. By experimental methods it is possible to induce lesions of tuberculosis in the striped gopher (*Citellus tridecemlineatus*) with each of the three bacillary forms of *Mycobacterium tuberculosis*. The

animals are more susceptible to organisms of bovine and human origin than to those derived from avian sources. The route of inoculation has a significant bearing on the induction of subsequent lesions, the intravenous route being the most effective.

REFERENCES

1. Hewetson, S. W. The gopher: a possible substitute for the guinea-pig. A preliminary report. *Med. News*, 1905, **87**, 824.
2. Feldman, W. H. The pathogenicity for dogs of bacilli of avian tuberculosis. *J. Am. Vet. M. A.*, 1930, **76**, 399.

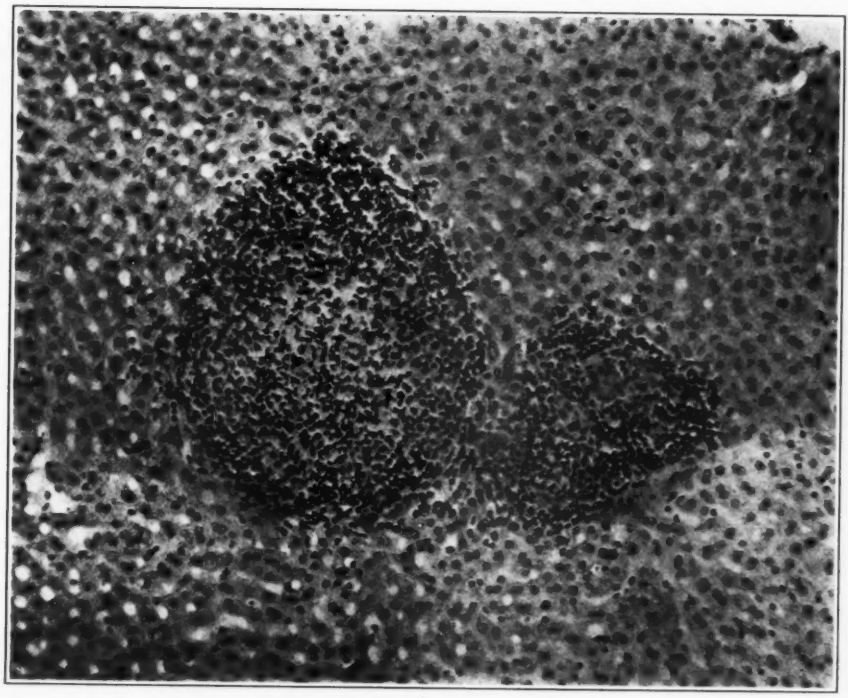
DESCRIPTION OF PLATES

PLATE 28

FIG. 1. Lungs of Gopher 11. Extensive tuberculous involvement. The animal died seventy-eight days after receiving intraperitoneally organisms of tuberculosis of human origin.

FIG. 2. Tuberculous foci in the liver of Gopher 7. The animal was killed sixty-six days after the intravenous injection of bacteria of avian origin. × 180.

1



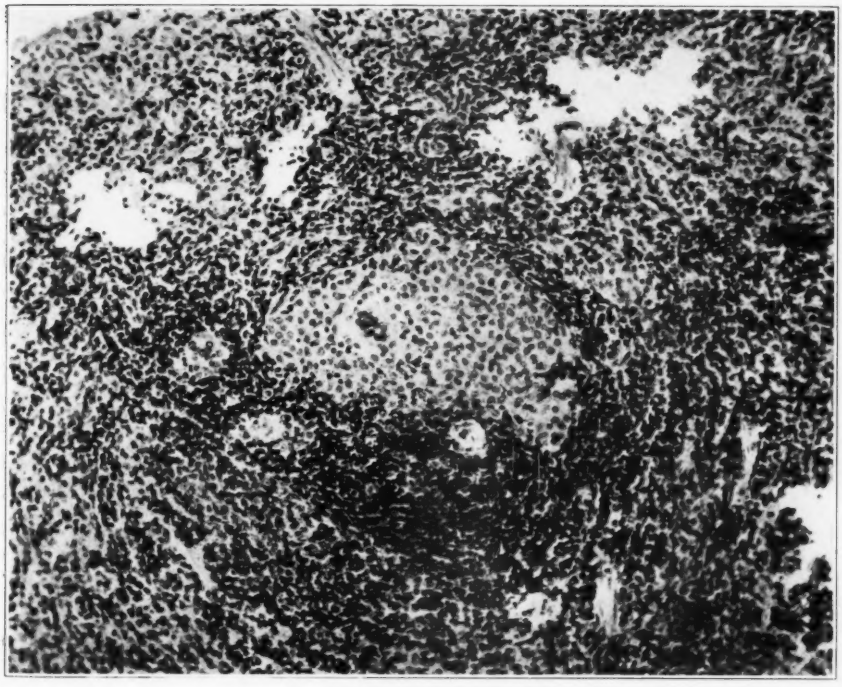
2

Feldman Susceptibility of Gopher to Tuberculosis

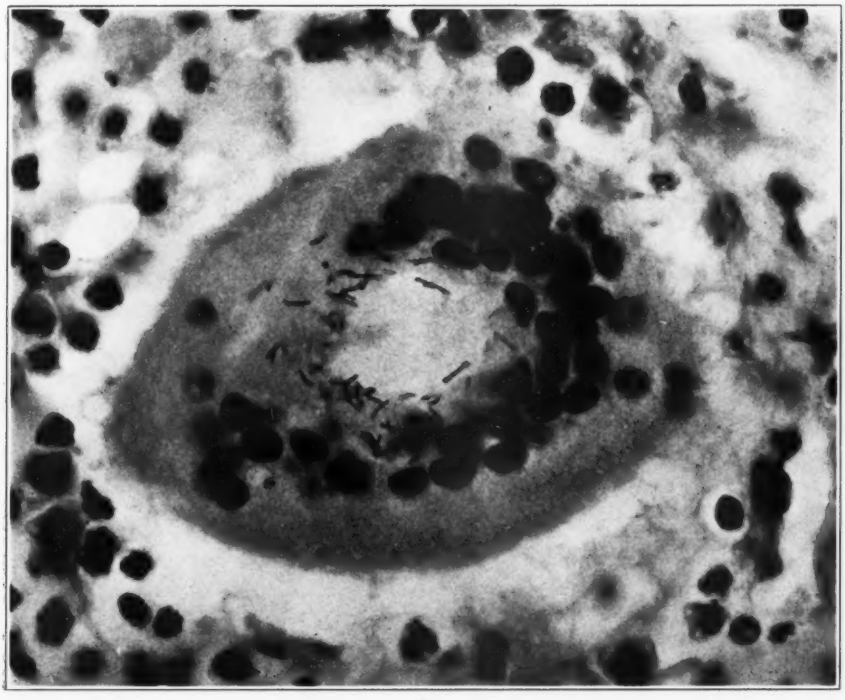
PLATE 20

FIG. 3. Collection of epithelioid cells replacing the normal elements of a splenic nodule of Gopher 7. The animal was killed sixty-six days after receiving an intravenous injection of bacteria of avian tuberculosis. × 150.

FIG. 4. Large tuberculous giant cell containing many acid-fast bacteria, from a hepatic lesion in Gopher 11. The animal died seventy-eight days subsequent to the intraperitoneal injection of *Mycobacterium tuberculosis* of human origin. × 1100.



3



4

Feldman Susceptibility of Gopher to Tuberculosis

THE PATHOLOGICAL CHANGES FOLLOWING EXPERIMENTAL EXPOSURE OF DOGS TO MYCOBACTERIUM TUBERCULOSIS OF AVIAN ORIGIN *

WILLIAM H. FELDMAN, D.V.M., M.Sc.

(From the Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minn.)

Although spontaneous tuberculous infections due to the bacillus of avian tuberculosis have been observed in many of the common mammals, including swine, horses, cattle and sheep, I have been unable to find in the literature reviewed a single instance in which the dog was affected. Since there are many opportunities for the average farm dog to become infected spontaneously with the avian form of the disease, his apparent failure to do so must be due to rather definite factors, but of an intangible nature.

In a series of experiments previously reported [1] it was shown that although the dog was extremely resistant to bacilli of avian tuberculosis by ordinary means of exposure, organisms injected directly into the cerebral substance initiated a definite tuberculous infection which was usually rapidly fatal. It was also noted that when large numbers of the organisms were introduced directly into the blood stream, tuberculous lesions of a mild type occasionally occurred. In my previous report I did not give a detailed account of the pathological changes observed, and therefore, these will be considered here.

The infective material used in these experiments consisted either of emulsions prepared from the liver and spleen of chickens which had died as a consequence of tuberculous infection, or of bacterial suspensions of bacilli which had been isolated in our laboratory by the method of Corper and Uyei, [2, 3] from spontaneous cases of avian tuberculosis. The experiments which were productive of definite lesions of a tuberculous character were those in which the organisms were injected intravenously or intracerebrally.

The jugular vein of ten adult dogs was injected with bacilli of avian tuberculosis. Only one of the animals died spontaneously; the others were killed for autopsy over a period of a hundred days to a

* Received for publication December 12, 1930.

year after injection. In only one of the animals were lesions demonstrated which could be considered of a definitely tuberculous nature. In three others, however, a few lesions were observed which were histologically identical with those of tuberculosis, although acid-fast organisms were not found among the cells constituting the respective lesions. The lesions in three of these four animals were confined to the liver and were of microscopic dimensions. With one possible exception, in none of the ten animals were lesions found grossly or microscopically in the lungs, spleen or kidneys.

Histologically the lesions in the liver of the animals were essentially the same after intravenous injections. They consisted of irregular, ovoid to spherical masses of monocytic cells, distributed among which were a few cells of a lymphoid character (Fig. 1). The larger masses occurred in the connective tissue elements surrounding the portal canals, whereas the smaller foci were present among the cells of several of the lobules of the liver. None of the separate lesions was in any sense large, and although the lesions were sharply separated from the adjacent parenchymatous cells, they were not divided from them by interposing elements. The zone of contact was devoid of a definite cellular response which might be interpreted as an expression of resistance. The Langhans' type of giant cell was not apparent in any of the material from the liver, nor were definite necrobiotic changes seen. Although the lesions in the liver of three of the dogs failed to reveal acid-fast organisms by appropriate staining methods, organisms morphologically indistinguishable from *Mycobacterium tuberculosis* were demonstrated in the fourth animal of the series. In addition, the organisms were successfully cultivated from the liver by the method of Corper and Uyei,[2,3] and typical lesions of tuberculosis developed in a chicken following intravenous injection with a portion of an emulsion prepared from the liver of this dog. The lesions in this liver were much more numerous than in the liver of the other three dogs and they appeared to be of a progressive nature, in contradistinction to the more or less quiescent appearance of the others.

In some of the sections prepared from the lung of the dog whose liver was so extensively involved, a definite tubercle-like collection of monocytic cells was seen. The failure to demonstrate bacilli of tuberculosis among the cells of these lesions leaves their true nature one of conjecture. Morphologically, however, they simulated rather

closely lesions induced in the lung of another dog as a consequence of an injection of bacilli of tuberculosis of human origin.

By far the most significant lesions were observed in the tissues of the dogs that were exposed to bacilli of avian tuberculosis by the intracerebral method of inoculation. In this group there were six animals, each of which received intracerebrally 1 cc. of a heavy saline suspension of *Mycobacterium tuberculosis* of proved avian type. Three different strains of the organism were used and two dogs were injected with each strain. All the dogs given intracerebral injections died seventeen to twenty-nine days after the inoculation, and definite tuberculous lesions were readily demonstrated in the tissues of each dog.

In the brains of each of the dogs which had been given intracerebral injections there was no evidence of pyogenic infection being a contributory factor in the death of the animal. The operative wounds had healed and the frequent and regular recording of the temperature of each of the animals failed to disclose a febrile state such as one might expect if an infection had been present due to the ordinary pyogenic microörganisms.

On the removal of the part of the cranium necessary for the removal of the brain, more or less severe meningitis was observed. The dura mater was commonly adherent to the cranium at the point of the perforation incidental to the injection and a major portion of the membrane was usually congested. In a few of the animals the dura mater was adherent to the pia mater, particularly over the cerebral hemisphere receiving the inoculation, and the latter membrane was in a state of congestion in all instances. Hydrocephalus was not observed; in fact, the amount of fluid present was, if anything, considerably less than normal. The presence of acid-fast bacilli was demonstrated in smears made from the pia mater of the brains of all the dogs, and in cases in which cultures were attempted the results were successful.

Blocks were obtained for histological study from several different portions of each brain. Sections were stained with hematoxylin and eosin and carbol fuchsin and hematoxylin, the latter for the purpose of demonstrating the presence of acid-fast bacteria.

Microscopically a vigorous monocytic proliferation was present which was so consistent throughout all of the material studied as to warrant the conclusion that the reaction had been induced by an

excitant common to all. The lesions were distributed for the most part under the pia mater of the surface of the brain and in the sulci. In a few instances they were also observed as discrete foci in the substance of the cerebrum.

Anatomically the lesions of the pia mater seemed to have had their inception in the region immediately adjacent to the blood vessels. The lesions expanded diffusely in a perivascular manner, and in not a few instances perivascular collections of monocytic cells were present in the depth of the cerebral tissue. All the blood vessels were highly congested, but parenchymatous retrograde changes of the cerebrum or cerebellum other than those in immediate contact with the specific cellular reactions were not apparent.

Examined minutely, the respective cellular reactions were of a diffuse character with no demonstrable tendency toward the formation of tubercles (Fig. 2). Most of the cells were of the usual monocytic character, disposed in compact formation, and the only discernible stroma was the occasional remnant of the preëxistent connective tissue. Occasionally mitotic division of the monocytic cells was seen. A few small lymphocytic cells were distributed promiscuously among the other elements, but polymorphonuclear leukocytes were absent.

In the sections appropriately stained, many acid-fast bacteria morphologically identical with *Mycobacterium tuberculosis* were present among the cells of practically all of the lesions studied. Many of these appeared singly with no significant relation to the cells constituting the lesions, whereas many others appeared in clumps which often occupied the cytoplasm of a monocytic cell. Many of the phagocytosed bacteria were smaller than normal and the remains of many were represented by coccoid forms. For the most part, acid-fast bacteria were rather numerous throughout the tissues of the brain.

The Langhans' type of giant cell was not observed, although a careful search for it was made. The central portion of an occasional lesion was undergoing caseation necrosis although this type of retrogression was by no means commonly present. Calcification was not observed.

A study was made of the spinal cord of one of the dogs. The animal had succumbed twenty-four days subsequent to the intracerebral injection of bacilli of avian tuberculosis and sections were

obtained from the cervical, thoracic and sacral portions of the spinal cord. Although demonstrable involvement was not observed in the tissues from the sacral and thoracic portions examined, there were unquestionable lesions in tissues obtained from the cervical portion. The lesions which involved the pia mater extended entirely around the cord in a diffuse manner, but in no instance was the substance of the spinal cord violated. Solitary tubercles were not apparent (Fig. 3). The reactive elements constituting the lesions were similar to those concerned in the lesions of the brain except that the monocytic cells were less compactly arranged and between the cells there seemed to be a little fluid. Polymorphonuclear leukocytes were missing, although small lymphocytes were fairly numerous. As in the lesions of the brain, giant cells were not seen nor were there any of the usual criteria of a reparative process. Acid-fast bacilli, although readily demonstrable, were not numerous. As far as could be determined, the dura mater was not implicated in the infective process.

Histological examination was made of the lungs, kidneys, pancreas, thyroid and suprarenal glands of the six dogs that had been given intracerebral injections, but tuberculous lesions were not found. In the spleen of one dog a few acid-fast bacilli morphologically identical with *Mycobacterium tuberculosis* were observed, but only a slight monocytic reaction was observed.

The livers of all dogs in the series that were given intracerebral injections were found to possess definite tuberculous lesions. These consisted of multiple, discrete collections of monocytic cells in the substance of the various lobules of the liver (Fig. 4). The respective foci seemed to have had their inception in the walls of the sinusoids and the lesions varied in number in the respective animals from numerous and extensive foci to an occasional collection of monocytic cells. The majority of the lesions were vigorously proliferating and an occasional monocytic cell in mitosis could be seen. The lesions appeared to be enlarging expansively at the expense of the adjacent hepatic cells which were gradually being replaced. The remaining cells of the parenchyma, except those in immediate contact with the lesions which were atrophic, were not visibly altered although all of the blood channels were congested. In none of the involved livers was there the slightest evidence that the lesions had induced any inhibitory influence whatever on that part of the liver.

Neither giant cells nor polymorphonuclear leukocytes were present and only an occasional lymphocyte was seen. Necrotic changes in the lesions were not apparent. Acid-fast bacilli, although rather scarce, were capable of demonstration within the lesions. Cultures of *Mycobacterium tuberculosis* were obtained from several of the livers.

Comment

The methods used in inducing experimental infection of the dog with bacilli of avian tuberculosis have made it possible to obtain information concerning the manner in which the tissues of the dog respond to an infection by this organism. From the morphological point of view it has been possible, therefore, to study several factors of interest in the pathological concept of tuberculous infections.

The distribution of the lesions suggests the significance of the susceptibility of organs in the pathogenesis of tuberculosis, and at the same time leaves one without an adequate explanation of this little understood phenomenon. Although it was possible to establish a tuberculous involvement of the liver in dogs that were given injections either intracerebrally or intravenously with *Mycobacterium tuberculosis* of avian origin, the lungs seemed to escape the disease. There can be no reasonable doubt about the lungs having been exposed to the infective material by both routes of inoculation, particularly by the intravenous route in which the exposure was massive. Apparently, however, the residence of the microörganisms in this organ was not sufficiently prolonged to incite a cellular reaction demonstrable at the time of autopsy. The spleen likewise exhibited marked resistance to the infection.

Corper and Lurie [4] observed marked variability in the susceptibility of the various organs of the dog to human and bovine bacilli of tuberculosis, which they attributed to differences in the ability of the various phagocytic cells to destroy the offending organisms. They considered the extensive involvement of the liver as being due to the limited capacity of the Kupffer cells to destroy the bacteria, and the remarkable resistance of the spleen to the infection could be accounted for, they thought, by the ability of the "histiocytic elements of the spleen to effectually destroy the tubercle bacilli." The failure of the infective material in my experiments to establish definite lesions in the lungs may have been due to similar

factors as those advanced by Corper and Lurie for the spleen. The failure of the disease consistently to promote extensive lesions in the liver of all dogs given intravenous injections, and the occurrence of multiple lesions of a progressive character in the livers of all dogs exposed by intracerebral inoculation cannot, however, be explained on the basis of the defense capacity possessed by the Kupffer cells. In fact the number of organisms injected intravenously was infinitely greater in every instance than those injected by way of the brain, and yet with one exception the lesions in the liver following the intravenous injections were quiescent and atrophic. Since the intravenous injections were given by way of the jugular vein, the possibility of the phagocytic cells of the lung engulfing and incapacitating the majority of the organisms before they could reach the liver is perhaps worthy of mention, but devoid of proof. On the other hand, in the intracerebral injections, organisms which were primarily established in the brain eventually gave rise to tuberculous foci in the liver and if they were transported from the brain to the liver by way of the blood stream one might reasonably expect the lungs to react in a manner comparable to that suggested in the animals that were given intravenous injections. That the lungs of all animals were free of definite demonstrable tuberculous lesions at autopsy rather forcibly suggests the possession in these organs of a most formidable defensive mechanism against the bacillus of avian tuberculosis, which is inadequate in the case of the human or bovine form of *Mycobacterium tuberculosis*. The exact mechanism of this resistance is worthy of further study.

Although cellular units identical with the typical miliary tubercle were not observed in the study of the various tissues of the dog in which there were lesions, this difference may have been due, at least in part, to the relatively immature age of the respective lesions in the majority of the animals. It should be noted, however, that there was very little structural difference between the lesions in the liver of the dog which died eighteen days after intracerebral exposure, and the lesions in the liver of the dog which was killed for autopsy a hundred days after an intravenous injection of the infective organisms. The lesions in both instances were essentially nothing more than compact, spherical or ovoid accumulations of monocytic cells. The cells failed to exhibit any definite tendency to assume an elongated form such as is commonly observed in the "epithelioid" cells of many

typical tubercles, and necrosis was infrequently observed. Total absence of giant cells of the usual Langhans' type was noted. Even in the occasional lesions of the brain which were undergoing caseation necrosis, giant cells were not observed. I do not believe that the age of the lesions is of first importance in the development of giant cells in tuberculous lesions since I have observed these structures in tubercles in a rabbit's liver eleven days after the injection of bacilli of avian tuberculosis. Even in spontaneous tuberculosis of the dog due to organisms of human origin, giant cells do not seem to be a part of the morphological reaction.[5] These observations suggest that the Langhans' type of giant cell is not a part of the cellular response in tuberculous infections in the dog.

Although the dog unquestionably possesses a stubborn resistance toward *Mycobacterium tuberculosis* of avian origin when exposed by ordinary means, the morphological data in this study indicate that once the infection becomes established the respective monocytic cells of the lesions exercise considerable inherent ability to withstand retrograde changes successfully. This was evidenced by the minimal amount of necrosis observed and the absence of calcification even in lesions which are definitely atrophic.

Summary and Conclusions

1. A detailed histopathological study was made of the tissues obtained from dogs which had been infected with bacilli of avian tuberculosis. Infection was accomplished by injecting the organisms directly into the brain in six animals, and an occasional infection was obtained by the introduction of large numbers of the organisms intravenously in ten animals.

2. In the dogs in which the infection was established by way of the brain, definite tuberculous lesions were found in the brain and liver in every instance. In only one animal was the spleen involved, and the lungs escaped demonstrable infection in each case. In the few animals in which the disease resulted subsequent to the intravenous route of inoculation, the lesions which were for the most part non-progressive were sharply limited to the liver. The information at hand seems inadequate to explain satisfactorily the consistent and extensive infection of the liver in the intracerebrally inoculated animals, in contradistinction to the infrequency of lesions in the

liver of the dogs inoculated intravenously. Likewise the failure of the disease to become manifest in the lungs is difficult of acceptable explanation and emphasizes the significance of the susceptibility of organs in the pathogenesis of tuberculosis.

3. The lesions induced were essentially circumscribed or diffuse accumulations of monocytic cells. As a rule, most of the lesions were progressive, and necrosis was not common. The apparent absence of giant cells would indicate that these structures are not a part of the histological response of the dog toward tuberculous infections.

4. Although the dog's brain offers a vulnerable portal of entry for the subsequent development of a tuberculous process by bacilli of avian tuberculosis, attempts to establish an infection with this organism by other means bring out the fact that the dog possesses an extremely formidable constitutional resistance to this form of the disease.

REFERENCES

1. Feldman, W. H. The pathogenicity for dogs of bacilli of avian tuberculosis. *J. Am. Vet. M. A.*, 1930, **76**, 399.

2. Corper, H. J. The certified diagnosis of tuberculosis. Practical evaluation of new method for cultivating tubercle bacilli for diagnostic purposes. *J. A. M. A.*, 1928, **91**, 371.

3. Corper, H. J., and Uyei, Nao. The cultivation of tubercle bacilli. An improved method for isolation from tuberculous materials. *J. Lab. & Clin. Med.*, 1928, **13**, 469.

4. Corper, H. J., and Lurie, M. B. The variability of localization of tuberculosis in the organs of different animals. I. Quantitative relations in the rabbit, guinea pig, dog and monkey. *Am. Rev. Tuberc.*, 1926, **14**, 662.
II. The importance of the distribution of tubercle bacilli as concerns differences of susceptibility of the organs. *Am. Rev. Tuberc.*, 1926, **14**, 680.
IV. The cellular factor in the susceptibility of the various organs. *Am. Rev. Tuberc.*, 1927, **15**, 237.

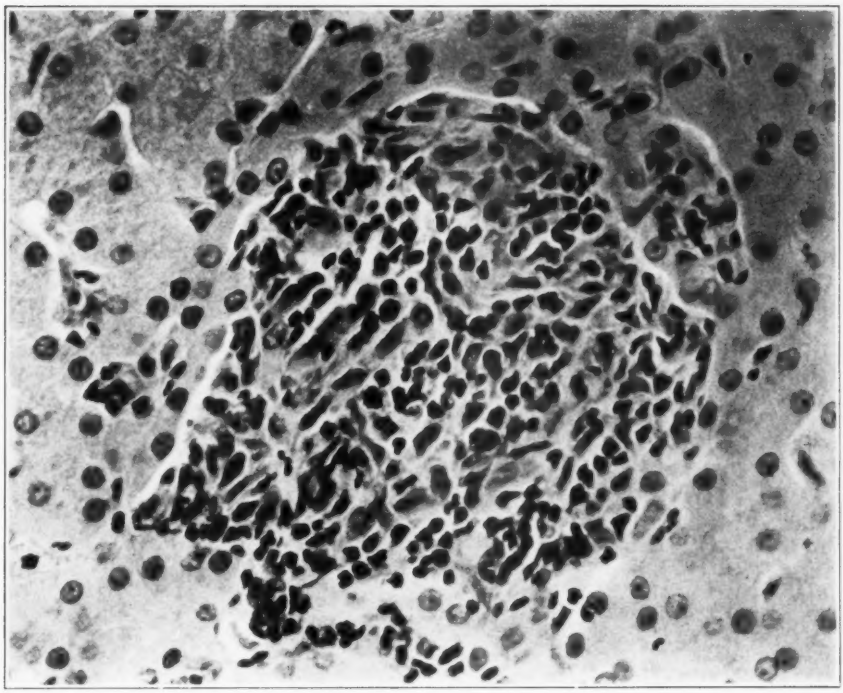
5. Feldman, W. H. Spontaneous tuberculosis in a dog. *Proc. Staff Meet., Mayo Clin.*, 1930, **5**, 78.

DESCRIPTION OF PLATES

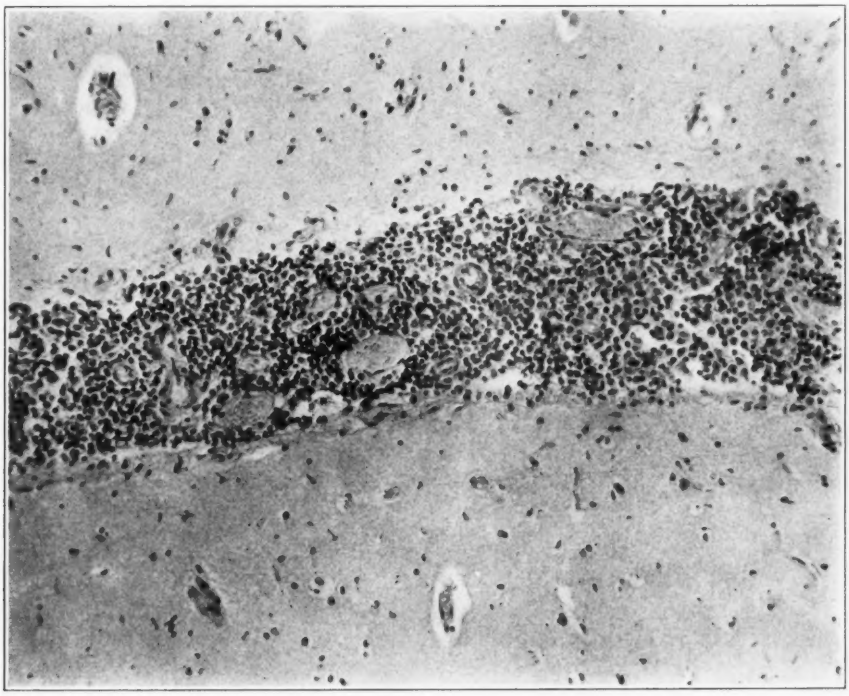
PLATE 30

FIG. 1. Accumulation of monocytic cells, many of which are definitely atrophic, in the liver of a dog killed for autopsy two hundred twenty-six days after the intravenous injection of bacilli of avian tuberculosis. Absence of encapsulating elements may be noted. × 660.

FIG. 2. Tuberculous lesion in the sulcus of the brain of a dog which died seventeen days after the intracerebral injection of bacilli of avian tuberculosis. The diffuse character of the reaction with no tendency toward the formation of tubercles may be noted. A few lymphocytes are present. Acid-fast bacilli were observed among the cells of the lesion. × 100.



1

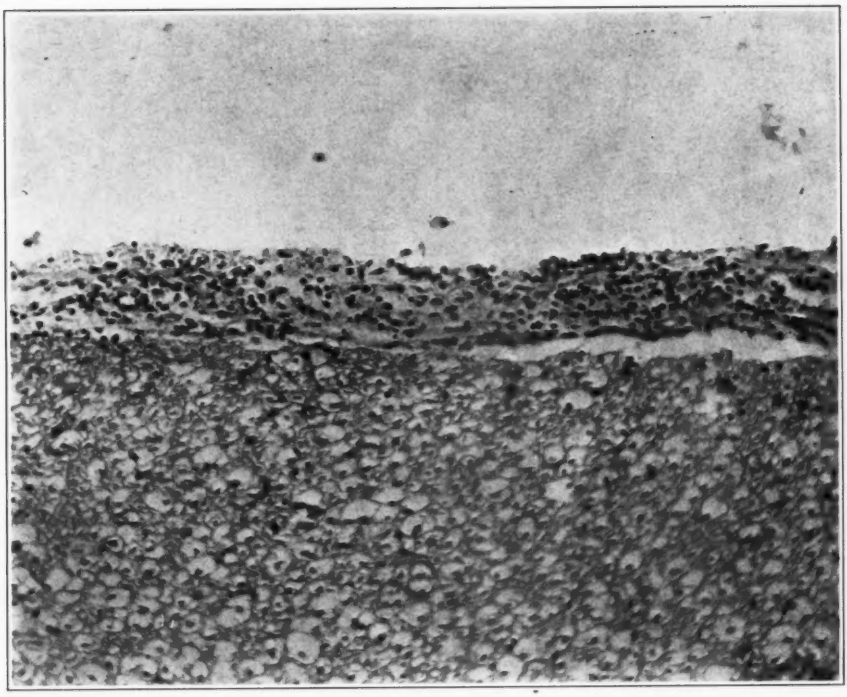


2

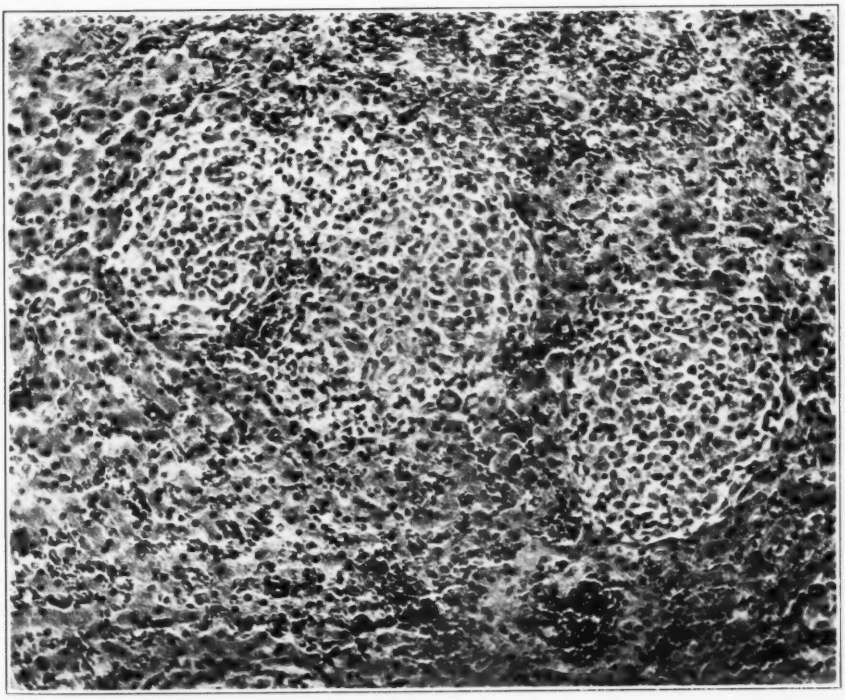
PLATE 31

FIG. 3. Tuberculous meningitis of the middle cervical portion of the spinal cord of a dog which died twenty-four days after the intracerebral injection of bacilli of avian tuberculosis. The cellular reaction was limited to the pia mater. Acid-fast organisms were demonstrated in the lesion. × 200.

FIG. 4. Multiple tuberculous foci in the liver of a dog which died eighteen days after receiving an intracerebral injection of *Mycobacterium tuberculosis* of avian origin. The lesions were progressive and were promiscuously distributed throughout the organ. × 220.



3



4

PRIMARY NEOPLASM OF HEART VALVE *

CHARLES F. BRANCH, M.D.

(From the Pathological Laboratory of the Massachusetts Memorial Hospital, and the Boston University School of Medicine, Boston, Mass.)

Primary neoplasms of the heart valves, although usually of no clinical importance, are of interest because of their great rarity. Less than a score have been reported to date.

We may note here that myxomas have been reported by Debove,[1] Djewitzky,[2] Guth,[3] Leonhardt,[4] and Ribbert.[5] Fibromas have been reported by Curtis,[6] Forel,[7] Reitmann [8] and Ribbert, while Forel claims to have found the only endothelioma to date. These authors have carefully recorded all of their findings in these cases and each insists that his tumor is a true neoplastic growth. These tumors are not to be confused with the hemorrhagic nodules occasionally noted on the heart valves of infants. Koechlin [9] was the first to note and question the origin and classification of these "Blutknötchen." Since that time many authors have reported this type of growth, both in infants and adults. The papers of Wendel [10] and Wegelin [11] form a comprehensive survey of this particular entity.

Lambl [12] in 1856 wrote of papillary excrescences on the aortic valve; these were perhaps of the true tumor group, although some authors seem to feel that they represented only sclerosed hemorrhagic nodules.

It seems from a scrutiny of the many detailed reports that some of the alleged neoplasms perhaps more rightfully were persistent blood nodules, while it is equally possible that a few of the latter, particularly Lambl's group, were true neoplasms.

The tumor I wish to report was discovered in the routine course of an autopsy performed on a woman, 35 years of age, who had a fatal secondary hemorrhage following hysterectomy. No essential pathology, other than the hemorrhage, was present.

The heart weighed 275 gm., and measured 13 by 9 by 4.5 cm. The epicardium was smooth, glistening and covered a small amount of subepicardial fat. The myocardium was firm, pale red-brown, and

* Received for publication January 27, 1931.

not remarkable in gross. The endocardium was smooth and glistening. With the exception of the tricuspid, the valves were normal as were also the coronaries. Foramen ovale and ductus arteriosus were closed. The tricuspid valve (Fig. 1) presented on its upper surface at the margin, a small, pedunculated growth composed of many fibrous, yet delicate, lace-like villous processes, growing about the pedicle in a tree-like manner.

Microscopically, as can be seen in Fig. 2, this growth consists of multiple branching papillae which contain no blood vessels. Every branch or stalk of tissue is composed of four different structures. In the center is a single or compound core of closely compacted wavy bundles of delicate collagen fibrils. Only a few fibroblasts are present among them. These central cores are surrounded by a thin layer of loose collagenous material containing relatively more fibroblasts. Next comes a homogeneous layer of varying thickness in which a small number of cells often with branching cytoplasmic processes are present. In places the two outer layers are more or less intimately fused together. On the outside, covering every stalk, is a layer of endothelial cells, some of which are multinucleated and contain as many as eight nuclei.

Weigert's elastic tissue stain shows fairly numerous fibrils in places, both in the central core and more abundantly in the loose connective tissue layer covering it. None can be found in the homogeneous layer. Mallory's phosphotungstic acid hematoxylin stain shows fibroglia fibrils to be present fairly numerously in the loose connective tissue layer, but also to some extent in the central cores and in the homogeneous layer. A silver stain demonstrates reticulum fibrils in the two outer layers.

There is no evidence anywhere of old fibrin undergoing organization.

In a few places a little fresh fibrin and small numbers of red blood corpuscles occur in clefts in the two outer layers. A few leucocytes are also sometimes present. These elements would seem to have been included in these layers during the process of growth.

Underneath the endothelium lining the valve is a homogeneous layer like that covering the papillary stalks of the tumor. It abuts directly on a rather dense layer of connective tissue. In it in places are delicate fibroglia fibrils.

In conclusion it may be helpful as well as interesting to summarize

the few statistics that can be compiled at this time. As far as we can learn there are now on record thirteen cases of primary neoplasms of the heart valves, excluding the blood nodule group and Lambl's papillary excrescences. They are all small, benign and clinically unimportant. The sex was reported in eight cases; seven were females. The youngest was 22 years of age, the oldest 83. Incidence of occurrence on the valves was as follows: tricuspid valve 6, mitral valve 3, pulmonic valve 2, aortic valve 2. Seven were avascular in nature. Tumor types encountered were myxyoma 6, fibroma 6, endothelioma 1.

Summary and Conclusions

A papillary branching fibroma of the tricuspid valve is described. The tumor, like the valve, contains no blood vessels. Every papillary stalk of the tumor is composed of a single or compound core of dense collagen fibrils surrounded by loose connective tissue, outside of which is a layer of homogeneous material covered by endothelium. The tumor seems to show a gradual growth and transformation of fibroblasts, starting in the homogeneous layer and ending in dense fibrous tissue. A similar homogeneous layer occurs beneath the endothelium lining the valve.

REFERENCES

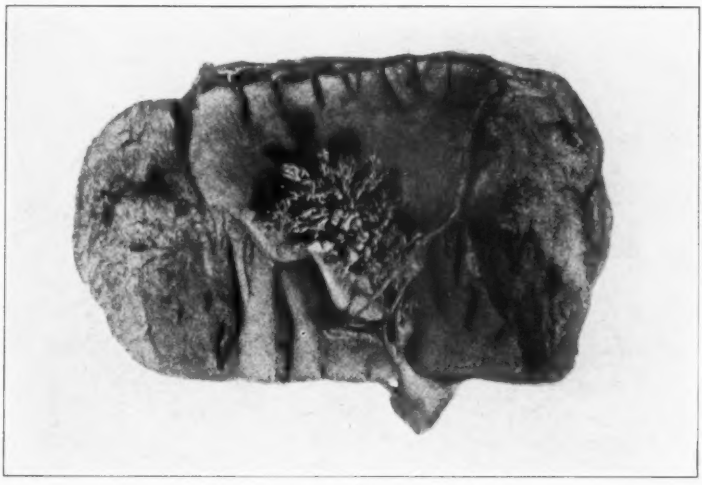
1. Debove. *Bull. et mém. Soc. anat. de Paris*, 1873, **48**, 295.
2. Djewitzky, W. S. *Virchows Arch. f. path. Anat.*, 1906, **185**, 195.
3. Guth, H. *Prag. med. Wchnschr.*, 1898, **23**, 85.
4. Leonhardt, A. *Virchows Arch. f. path. Anat.*, 1905, **181**, 347.
5. Ribbert, H. Geschwulstlehre für Ärzte und Studierende, 1904, 233.
6. Curtis, M. B. *Arch. de physiol. norm. et path.*, 1871–72, **4**, 262.
7. Forel, F. C. *Internat. Clin.*, 1919, S. iv, 147.
8. Reitmann, K. *Ztschr. f. Heilk.*, 1905, **26**, 67.
9. Koechlin, E. *Frankfurt. Ztschr. f. Path.*, 1908, **2**, 295.
10. Wendel, H. *Frankfurt. Ztschr. f. Path.*, 1917, **20**, 299.
11. Wegelin, C. *Frankfurt. Ztschr. f. Path.*, 1908–09, **2**, 411.
12. Lambl. *Wien. med. Wchnschr.*, 1856, **6**, 243.

DESCRIPTION OF PLATE

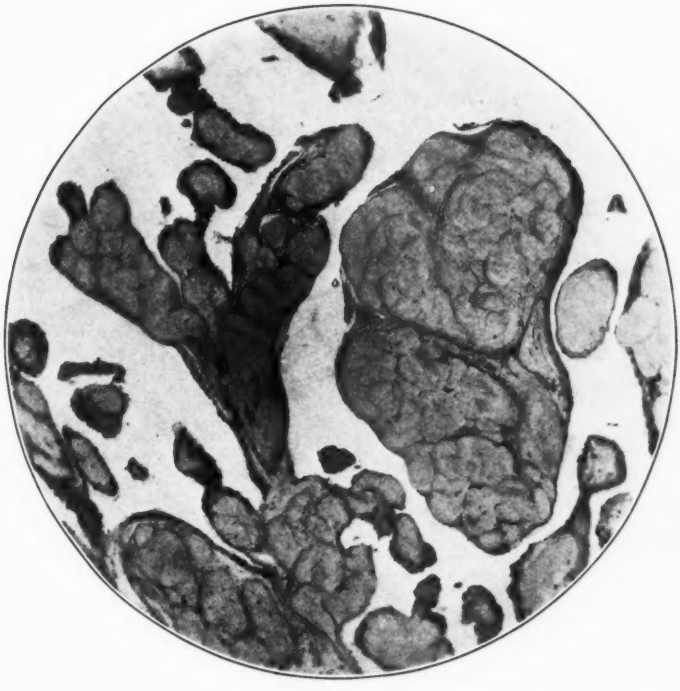
PLATE 32

FIG. 1. Tricuspid valve showing on its upper surface a delicate branching villous tumor.

FIG. 2. Branching stalks of tumor tissue composed of single and compound cores of dense collagen surrounded by loose connective tissue and a homogeneous layer covered with endothelial cells. × 40.



1



2

Branch

Primary Neoplasm of Heart Valve

CHONDROSARCOMA WITH INTRAVASCULAR GROWTH AND TUMOR EMBOLI TO LUNGS *

SHIELDS WARREN, M.D.

(From the Laboratory of Pathology, New England Deaconess Hospital, and the Department of Pathology, Harvard Medical School, Boston, Mass.)

While the bizarre intravascular growths of chondrosarcoma have long been known,[1, 2] a case recently observed in this laboratory has sufficiently unusual features to warrant reporting it. There is rather marked similarity between this present case and one reported by Ernst,[3] an enchondroma originating in the twelfth thoracic vertebra, which had invaded the renal and suprarenal veins, the vena cava and the left ovarian vein. There were masses of the tumor in both pulmonary arteries, in the right a bifurcated mass of tumor tissue, whereas the one lodged in the left pulmonary artery had extended into the branches of the artery so that it had a hand-like appearance. There were no true metastases. In Ernst's article references to the earlier cases are given.

REPORT OF CASE

Clinical History: W. Y., a white male, 32 years of age, was admitted to the New England Deaconess Hospital May 20, 1930, under the care of Dr. Dwight L. Sisco, complaining of pain and soreness in the lower back. Three weeks before he had begun to have pain in the left gluteal region, which he thought was due to muscular lameness because of unusual exercise. On physical examination a walnut-sized nodule was palpable just above the left sacro-iliac joint. The examination was otherwise negative. An abnormal rarefaction of bone about the left sacro-iliac joint was found on X-ray examination. The lungs were clear. May 24, 1930, under gas anesthesia, the tumor was explored by Dr. William A. Rogers and found to be a chondrosarcoma. July 5, 1930 the patient was transferred to the Palmer Memorial Hospital, under the care of Dr. E. L. Daland. A course of deep X-ray therapy was given. The patient steadily lost strength and had considerable pain. October 8, 1930, at 7 P.M. the patient had a sudden attack of pain in the precordial region, which was extremely intense and not controlled by morphia. The respirations increased slightly. An X-ray of the chest on this date showed diffuse clouding of the lung in the third left interspace, which suggested a focus of metastasis. October 11, 1930, physical ex-

* Received for publication January 21, 1931.

amination failed to elucidate any clearly defined focus of consolidation, although there was some dullness in the left upper chest. The fever ranged from 101 to 104 F. The patient failed steadily and died, rather suddenly, without marked change in pulse or respiration, October 27, 1930, at 11.30 A.M. Autopsy was performed two hours later.

Postmortem Examination

Body: Is that of a well developed, somewhat emaciated, young, adult white male. There is a well healed scar 10 cm. long along posterior crest of left ileum. Whole left side of pelvis from sacrum posteriorly to symphysis pubis anteriorly is markedly larger than right. A large subcutaneous, thick, slightly fluctuant mass can be palpated beneath skin. There are several superficial ulcerations in skin over buttocks. Moderate pitting edema of left ankle and legs. Left thigh larger than right. Rigor mortis absent. No icterus or distention.

Head: Moderate growth of short black hair. Pupils equal, regular, 6 mm. in diameter. Ears and nose negative. Teeth in fair condition.

Primary Incision: Y type. Panniculus adiposus 1 cm. thick.

Peritoneal Cavity: Surfaces smooth and glistening. No adhesions. Sigmoid is pushed anteriorly by a large tumor mass filling left iliac fossa.

Appendix present, retrocecal and normal. Diaphragm fourth rib right, fourth interspace left.

Pleural Cavities: Right negative. No adhesions or free fluid. Left cavity contains a few easily broken adhesions between upper lobe and chest wall. No free fluid.

Pericardial Cavity: Surfaces smooth and negative. Contains 30 cc. of clear yellow fluid.

Heart: Weight 290 gm. Epicardium negative. Opened *in situ*. Myocardium firm, dark reddish brown. Valves and endocardium negative except that pulmonary valve is composed of four cusps. Foramen ovale closed. Coronaries negative. Pulmonary artery clear but palpation detects a firm obstruction in left and right branches. Measurements: Tricuspid valve 12.5 cm., pulmonary valve 6.5 cm., mitral valve 8 cm., aortic valve 6.5 cm., left ventricle 1 cm., right ventricle 0.2 cm.

Lungs: Left lung: surface shows a roughened focus on upper lobe, adherent to parietal pleura. Beneath this is a rounded, markedly

resilient focus. On section this is found to be filled with air. Cavity entered measures 5 cm. in diameter and inner wall is roughened and covered with greenish, foul, purulent exudate. Wall of cavity 3 to 4 mm. thick. Three similar smaller cavities found in surrounding lung tissue. Left lower lobe negative. Left pulmonary artery completely occluded by a whitish gray, firm, cartilaginous mass which extends and ramifies to smaller branches of pulmonary artery in left and lower lobes. This is somewhat adherent to walls of vessels. Some of smaller branches of right pulmonary artery contain bits of whitish, cartilaginous material similar to that found in left pulmonary artery.

Right lung: contains several firm nodules up to 2 cm. in diameter, which on section show a firm, pale gray, glistening, inelastic structure. These are close beside or involving branches of the pulmonary artery.

Spleen: Weight 210 gm. Capsule smooth and dark reddish. Cut surface rather firm and dark reddish with distinct markings. Almost no pulp is scraped away.

Pancreas: Weight 60 gm. Normal in size and consistency. On section pale gray and elastic with normal lobulations.

Gastro-Intestinal Tract: Negative.

Liver: Weight 2080 gm. Surface reddish brown and smooth. On section, edges evert slightly. Cut surface chocolate brown with distinct lobulations and slightly congested central areas. Gall-bladder negative. Bile ducts patent. No stones present.

Adrenals: Negative.

Kidneys: Weight, right 290 gm., left 290 gm. Capsule strips easily from an irregular, dark reddish gray surface mottled with numerous yellowish foci measuring 2 to 4 mm. in diameter. Section of right kidney reveals a slightly dilated pelvis filled with thick, creamy, yellowish pus. Pelvic mucosa markedly injected. Cortex and medulla contain numerous discrete yellowish foci. There is poor differentiation between cortex and medulla.

Left kidney: on section similar to right, but contains fewer abscesses.

Ureters: Both are dilated and measure about 0.5 cm. in diameter. Filled with thick, creamy, yellowish, purulent material.

Bladder: Rather firmly contracted. Section reveals a dark reddish, markedly injected and roughened mucosa. Bladder wall

thickened, measuring 1 to 1.3 cm. in diameter and composed of whitish gray, firm, fibrous material.

Prostate: Negative.

External Genitalia: Negative.

Lymph Nodes: Along course of abdominal aorta are several enlarged nodes 1.5 cm. long, which on section are pale gray, firm and somewhat inelastic.

Veins: Inferior vena cava just above junction of iliac vein is blocked by a whitish gray, firm, cartilaginous mass which continues into smaller and larger tributaries of inferior vena cava, especially on left side. This growth is slightly adherent to vessel walls. On left side it extends as far as saphenous junction with femoral vein and into hypogastric vein. The latter is surrounded by tumor tissue. Right common iliac vein contains similar material as well as a slight amount of ante mortem thrombus.

Aorta: A few foci of atheromatous change.

Tumor: Beneath iliopsoas muscle in left iliac fossa is a large oval tumor measuring 17 cm. long and 10 cm. in diameter, extending from level of fifth lumbar vertebra above, to beneath the inguinal ligament below. This mass is firmly fixed and slightly fluctuant. Section through mass reveals a coarsely honeycombed, whitish gray structure with cavities filled with a thick, mucoid, yellowish brown fluid. Center of mass markedly cystic. Exact posterior limits of this mass cannot be determined but it involves sacrum, left side of bony pelvis, but not lumbar vertebrae.

Anatomical Diagnoses: Chondrosarcoma of left sacro-iliac synchondrosis with extension into common iliac veins, left femoral and left hypogastric veins and inferior vena cava; metastases to lungs; tumor embolus, left pulmonary artery; pyonephrosis, bilateral; pyelonephritis, bilateral; multiple abscesses of kidneys and left lung; pyo-ureter, bilateral; chronic cystitis; chronic passive congestion of liver; anomaly of pulmonary valve; edema of left leg; decubiti.

Microscopic Examination

Heart: Slight increase in connective tissue between muscle bundles.

Lung: Vessels are congested. In most areas the alveolar walls are somewhat broken near pleural surface. One section shows a small

necrotic area. Considerable fibrin, red blood cells and polymorphonuclear leucocytes in surrounding area. Pleural surface thickened by old granulation tissue. In some foci there is considerable anthracosis. Moderately large focus of lung tissue replaced by mass of well differentiated cartilage cells with considerable amount of matrix, some of which has undergone cystic degeneration. Considerable portion of mass is apparently within a blood vessel and some fibrin and red blood cells are present near the periphery. The tumor cells near the periphery are relatively undifferentiated, being of a plump spindle shape with only slight amount of intercellular substance. Mitoses are not infrequently seen. The surrounding lung tissue is compressed.

Spleen: Shows some congestion of sinusoids. Polymorphonuclear leucocytes are quite numerous in sinusoids.

Pancreas: Islet cells and acinar cells stain clearly. Lobules appear normal.

Liver: Sinusoids dilated and liver cords show some compression. In some areas liver cell nuclei are pyknotic. Cytoplasm is swollen and has a granular appearance.

Kidney: Numerous foci of polymorphonuclear leucocytes with necrotic centers are found. Glomerular tufts are congested in places and infiltrated with occasional polymorphonuclear leucocytes. Bowman's capsule distended and in many places red blood cells and mononuclear leucocytes are found in intracapsular space. Tubule cells show granular degeneration. Many nuclei are pyknotic. Tubules in many areas are filled with red blood cells. In a few areas near cortex there is a diffuse infiltration of interstitial tissue with polymorphonuclear leucocytes, mononuclear leucocytes and lymphocytes.

Adrenal: There is some necrosis in zona fasciculata of adrenal. Considerable congestion of vessels.

Bladder: Muscle wall appears normal. Mucosa is thickened and submucosa is infiltrated with lymphocytes and polymorphonuclear leucocytes in places. No evidence of tumor growth in this section.

Tumor: Bulk of tumor consists of somewhat atypical cartilage cells varying markedly in size, embedded in a considerable amount of matrix. Portions of matrix show extensive foci of cystic degeneration. Toward periphery numerous plump spindle-shaped, rounded cells varying markedly in size with fairly numerous mitoses and

scattered tumor giant cells. In a few foci a considerable amount of hemosiderin is present. One point in section shows a large vein almost completely filled by mass of tumor tissue, which is not adherent to the vessel wall except at one or two points. The intravascular portion of the growth does not vary in character from the extravascular. In several places apparently intact nerves are seen passing through the tumor. The tumor tissue itself is relatively avascular, except at the periphery.

Lymph Node: Section through aortic lymph node shows an increase in connective tissue in lymphoid follicles. No tumor cells are observed. Lumbar nodes show quite marked increase in connective tissue.

Aorta: Section examined shows slight amount of lipoid material in subintimal layer.

Discussion

The extent of intravascular growth of this tumor is extraordinary. Apparently the tumor gained access to the venous system through the branches of the left hypogastric vein and from there readily grew into the common iliac vein, extending on the one hand up into the vena cava and on the other hand down the left external iliac vein into the femoral vein. The intravascular tumor mass was of interest in that it lay, for the large part, free within the lumen of the vessel, being only slightly adherent. The cells of the tumor within the veins were in the main well differentiated and produced a very large amount of cartilage matrix.

Apparently on October 8, so far as can be judged from the clinical history, a considerable portion of the tumor mass broke away from the mass within the vena cava and was swept by the blood stream through the right heart into the left pulmonary artery, where it lodged. Apparently not disconcerted by this sudden change of locus, it continued its growth in various branches of the pulmonary artery until death of the host. In curious contrast to the minute fragments of the tumor which gave rise to typical pulmonary metastases with destruction of the lung parenchyma, the outgrowths of tumor tissue from this large embolic mass did not invade the lung tissue.

SUMMARY

A case of chondrosarcoma with extensive intravascular growth and tumor emboli in the pulmonary circulation is described.

REFERENCES

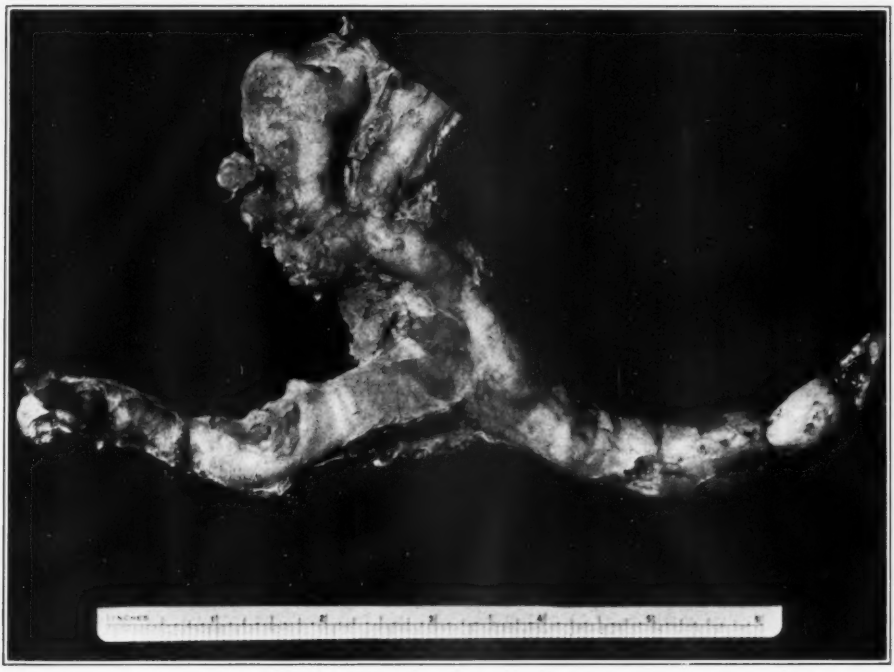
1. Virchow, R. *Virchows Arch. f. path. Anat.*, 1855, **8**, 404.
2. von Biesiadecki, A. *Sitzungsb. d. k. Akad. Wissensch.*, 1868, **57**, 793.
3. Ernst, P. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1900, **28**, 255.

DESCRIPTION OF PLATE

PLATE 33

FIG. 1. Portion of main tumor mass above with tumor growing within internal iliac vein, shown continuous with tumor mass in left external iliac and femoral veins. Small projection at extreme left lay within the left saphenous vein. Portion to right lay within the common iliac vein and first 2 cm. of vena cava.

FIG. 2. Drawing, natural size, of tumor embolus from left pulmonary artery, showing extensive intravascular growth from embolic mass.



1

2

Warren Chondrosarcoma with Tumor Emboli to Lungs

HEMORRHAGIC PANCREATITIS *

REPORT OF TWO CASES IN WHICH GALLSTONES COULD NOT BE CONSIDERED ESSENTIAL ETIOLOGICAL FACTORS

VINCENT J. DARDINSKI, M.D.
FELLOW IN PATHOLOGY, THE MAYO FOUNDATION
(*From the Section on Pathological Anatomy, The Mayo Clinic, Rochester, Minn.*)

Review of the literature reveals that hemorrhagic pancreatitis was studied by Rokitansky [1] in 1861, by Klebs [2] in 1869 and by Fitz [3] in 1889. They gave no explanation as to the etiology of the condition. They did, however, bring out the fact that hemorrhage in the pancreas, except when due to severe abdominal trauma, was usually associated with disease of the gall-bladder and bile passages. Many other observers during this period reported single cases in which hemorrhage had been observed in the pancreas, but their reports are too inadequate and inconclusive to be of much value.

Any suggestion as to the etiology of this condition, however, was not mentioned until 1901, when Opie [4] published his first account of a case, showing the relationship between pancreatic necrosis and impaction of a calculus in the ampullary portion of the common bile duct. Following this observation other cases were reported in which similar conditions were associated with acute hemorrhagic pancreatitis. Numerous experiments then followed in which it was attempted to reproduce this type of lesion in animals. The result of these experiments was the discovery that apparently the changes sought could be produced by injection of a variety of irritating substances, such as zinc chloride, artificial gastric juice, hydrochloric, nitric and chromic acids in varying strengths, and solutions of bacterial toxins, into the duct of Wirsung. Various oils also have been used, and all of these produced the same results. Various bland substances, however, such as blood, blood serum, agar-agar, paraffin, and emulsions of starch did not produce these changes.

Hlava,[5] in 1898, suggested that in human subjects hyperacid gastric juice may be forced by antiperistalsis of the intestine into the pancreatic duct, thus producing hemorrhagic pancreatitis. Levin,[6]

* Received for publication January 2, 1931.

in 1907, showed that crushing of the pancreas is associated with occlusion of the blood vessels, and with changes which resemble those of hemorrhagic necrosis. Mallory,[7] on the other hand, explained the occlusion of the pancreatic blood vessels in hemorrhagic pancreatitis by the action of the pancreatic enzyme on the walls of the vessels which he said produced thrombi.

Mann and Giordano [8] in a study entitled "The bile factor in pancreatitis," found the anatomical arrangement of the common bile duct and pancreatic duct to be such that the possibility of gallstones producing obstruction at the outlet of the papilla, to form a continuous channel from the common bile duct into the pancreatic duct, was small. They found that each duct opened separately into the duodenum in 31 per cent of cases. In 45 per cent the ducts united 0.2 cm. from the apex of the ampulla of Vater. In 20 per cent the ducts united 0.3 cm. to 10 mm. from the apex of the ampulla of Vater. In 4 per cent the pancreatic duct was absent or was reduced to a fibrous cord. They concluded that obstruction is possible in 20 per cent of cases providing the stone is larger than 3 mm. in diameter. If less than 3 mm. in diameter the possibility of obstruction being caused is slight, because the outlet of the papilla is usually 2 to 3 mm. in diameter and the stone might pass through without much difficulty.

Grant,[9] in 1928, reported twelve cases of acute pancreatic necrosis. Eleven of the twelve patients were operated on. Nine had stones in the gall-bladder. One had a single stone in the ampulla and one had a normal gall-bladder and stones were not found in the extrahepatic biliary tract. The reports of autopsy are indefinite as to the condition of the pancreas and of the outlet of the common bile duct and pancreatic duct. Hemorrhage in the pancreas was mentioned in only two of these cases.

Holzapfel,[10] in a more recent report, reached conclusions similar to those of Mann and Giordano, that hemorrhagic pancreatitis due to gallstones is possible in 20 per cent of persons because of the anatomical arrangement of the terminal portions of the common bile duct and pancreatic duct. In 80 per cent he found the common bile duct and the pancreatic duct each to have a separate opening into the intestine, either in the same or separate papillae. He expressed the same opinion as Opie that bile enters the pancreatic duct and activates the zymogens, thus producing hemorrhagic necrosis.

Judging from the literature the majority of authors seem to be of the opinion that a gallstone impacted in the ampulla of Vater converts the pancreatic and common bile ducts into a continuous channel and thereby diverts the flow of bile into the duct of Wirsung. The interaction of the bile with the pancreatic juice is believed to liberate an enzyme, probably trypsin, which is responsible for the lymphatic and vascular changes. In the cases reported here the anatomical arrangement of the pancreatic and common bile ducts at their terminations, does not give support to this theory.

Report of Cases

Case 1. The history and examination in the case of a woman aged 57 years were suggestive of acute hemorrhagic pancreatitis.

At the postmortem examination the gall-bladder contained about 40 cc. of dark, greenish, viscid bile and more than 200 stones, varying in size from a diameter of 3 to 4 mm. to that of fine granules of sand. The wall was soft and slightly thickened, but grossly it did not show signs of active inflammation. The common bile duct contained several of these small stones, but was not dilated and did not show evidence of previous injury from stone (Fig. 1).

The pancreas weighed about 120 gm. and was increased in consistence throughout. On section two large hemorrhagic portions were found confined to the central portion of the gland. The pancreatic duct passed through these hemorrhagic regions. It was not dilated or obstructed and did not contain bile or other foreign substances. The common bile duct and the pancreatic duct each had a distinct and separate opening in the tip of the papilla (Fig. 2). There was no evidence that there had been obstruction at any time, for both ducts appeared to be normal at their outlets.

Microscopic examination of the pancreas revealed the usual appearance of hemorrhagic pancreatitis with destruction of pancreatic tissue. Numerous thrombi in various stages of organization were found in the veins.

Case 2. A woman, aged 57 years, consulted the clinic because of a carcinoma of the left breast. Hemorrhagic pancreatitis was not suspected either before or after the operation for this condition.

The postmortem examination revealed the peritoneum in the region of the pancreas, below the transverse mesocolon and along the

base of the mesentery, to be studded with small, yellowish white, opaque spots of fat necrosis. There was hemorrhagic discoloration beneath the peritoneum around the pancreas. The gall-bladder contained 50 cc. of bile and there was slight cholesterosis of its mucosa. Gallstones were not present. The common bile duct appeared normal throughout. There was no evidence of stones, strictures or injury due to stones. In the middle of the pancreas were hemorrhagic regions. There was also more or less diffuse hemorrhage throughout the remainder of the gland. There were marked deposits of adipose connective tissue in the head and tail of the gland. These revealed typical, small, yellowish, opaque spots of fat necrosis. The pancreatic duct appeared to be normal throughout. Its outlet into the intestine was found to be separate from, and 5 mm. distal to, the outlet of the common bile duct.

Microscopic study of the pancreatic sections disclosed essentially the same changes as those which were present in Case 1.

Comment and Summary

In these two cases impaction of gallstones at the ampulla of Vater could not be considered an etiological factor in producing hemorrhagic disease of the pancreas. In Case 1, as shown in Fig. 2, the common bile duct and the pancreatic duct each had a separate opening on the tip of the papilla. In Case 2, the outlet of the pancreatic duct was 5 mm. distal to that of the common bile duct. There is no possibility in either case that the obstruction at the outlet produced a continuous channel from the common bile duct into the pancreatic duct.

REFERENCES

1. Rokitansky, Carl. Lehrbuch der pathologischen Anatomie. Braumüller, Vienna, 1855–1861, **3**, 313.
2. Klebs, Edwin. Handbuch der pathologischen Anatomie. Hirschwald, Berlin, 1868–1880, **1**, 271.
3. Fitz, R. H. Acute pancreatitis. *M. Rec.*, 1889, **35**, 197.
4. Opie, E. L. The relation of cholelithiasis to disease of the pancreas and to fat necrosis. *Am. J. M. Sc.*, 1901, **121**, 27.
5. Hlava. Quoted by Katz, A., and Winkler, F. Experimentelle Studien über die Fettgewebsnecrose des Pankreas. *Arch. f. Verdauungskr.*, 1898, **4**, 289.

6. Levin, Isaac. Effects of experimental injuries of the pancreas. *J. Med. Res.*, 1907, **16**, 419.

7. Mallory, F. B. The Principles of Pathologic Histology. W. B. Saunders Co., Philadelphia, 1914, 525.

8. Mann, F. C., and Giordano, A. S. The bile factor in pancreatitis. *Arch. Surg.*, 1923, **6**, 1.

9. Grant, J. W. G. Acute necrosis of the pancreas: report of a series of cases. *Brit. M. J.*, 1928, **1**, 1101.

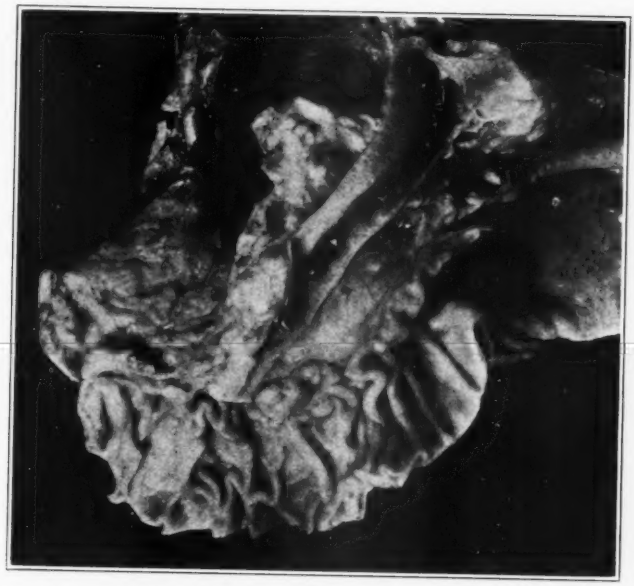
10. Holzapfel, Rudolf. Untersuchungen über die Ätiologie der Hämorrhagischen Pankreasnekrose. *Klin. Wchnschr.*, 1930, **9**, 596.

DESCRIPTION OF PLATE

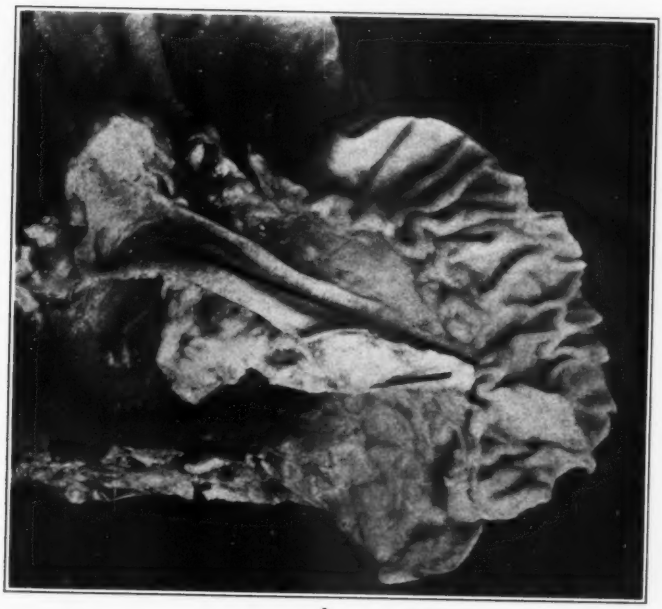
PLATE 34

FIG. 1. Case 1. The common bile duct, containing many gallstones. This specimen is the same as that shown in Fig. 2.

FIG. 2. Case 1. Probes in the common bile duct and pancreatic duct. The common bile duct and the pancreatic duct each opens separately into the outlet of the papilla.



1



2

Dardinski Hemorrhagic Pancreatitis

INTESTINAL ADENOMA IN SWINE *

H. E. Biester and L. H. Schwarte

(From the Department of Veterinary Investigation, Iowa State College, Ames, Iowa)

The ultimate result of epithelial regeneration following extensive destruction of mucosal tissue in infectious enteritis, and the degree of functional ability possessed by such regenerated epithelium seem of considerable importance in connection with the probability of recovery.

Kaufmann [1] notes that in severe cases of diphtheric enteritis associated with extensive destruction of tissue followed by the healing of ulcers, the remaining islands of mucosa may undergo glandular proliferation and produce intestinal polypi.

In spite of the greater opportunities for study, very little is known regarding the significance of adenomatous polypi in the intestines of domesticated animals. Petit and Germain [2,3] describe adenomatous growths in the stomach of the horse, due to the presence of *Habronema megastoma* Rud. 1819 (syn. *Spiroptera megastoma*) and *Trichostrongylus axei.*

Neumann [4] attributes intestinal tumors from the size of a pinhead to a small walnut to sclerostomes. However, he makes no reference to the histological structure of these growths. Bergmann [5] noted in the rectum of a 7 month's old pig, pea- to walnut-sized growths composed of simple or branched gland ducts. He found necrophorous organisms in the growths and believed that they could not be ruled out as the primary cause. Henk [6] reported adenomas in the large intestine of the pig, followed by hemorrhages. Numerous clinical and autopsy reports by European army veterinarians record the presence of adenomatous growths in the intestines of the horse, but their etiology was not studied. With the exception of a few case reports not readily accessible, no mention is made of intestinal adenomas of the pig.

In view of the extensive studies on this subject in the human, a brief consideration of some of the outstanding work seems indicated in connection with a study of adenomatous newgrowths in the in-

* Received for publication December 8, 1930.

testines of swine and their relation to extensive mucosal destruction and regeneration following bacterial or parasitic invasions. Port [7] reviewed a series of multiple intestinal polypoid adenomas and concluded that the condition could be traced to a familial disposition and that it is not a disease developing later.

Hauser [8] divides the adenomatous proliferations into two groups. The first consists of those in which there is a degeneration of the epithelium, loss of physiological function and an absence of goblet cells. The widespread nature of this form suggests to him a contagious or living form as the causal factor. As an example of this he cites the extensive bile duct proliferations in rabbits due to coccidia, but because protozoan forms were not consistently found throughout the intestinal adenomatous areas he ruled them out. He believes irritation may favor further growth in this group, as occurs at the curvatures of the intestine in diffuse intestinal adenomatosis, but that irritation does not act as a primary factor. His second group includes newgrowths in which the epithelium resembles that of a normal mucous membrane, and in which mucin production is increased. These he considers as a simple gland hypertrophy with increased function secondary to a chronic inflammatory process.

Ruffer [9] describes tumors in the intestine due to oxyuris. Eggs were found in these growths but apparently the tissues were not studied histologically. Borst [10] attributes intestinal adenomatous polypi to unused germ cells which remained latent during development, or to those which were misplaced. In his opinion irritation can arouse adenomatous growths but only in tissues disposed to tumor growth. Milton [11] records polypous formations in connection with bilharziosis. Hart,[12] on the basis of a described case, holds it probable that intestinal polypi originate as a result of irritation or stimulation by a carcinomatous metastasis. Doering [13] states that in most cases the etiology is not understood. He confirms the observation of others that it is a disease of the young and middle-aged. Funkenstein, Janowski and Askanazy, cited by Doering,[13] found no evidence of a relationship between this process and protozoa.

Verse [14] distinguishes between a congenital anomaly and a congenital disposition of the epithelium to increased proliferation similar to that found in xeroderma pigmentosum. Preparations containing hyperplastic glands in which goblet cells are being displaced by undifferentiated epithelium are submitted as evidence of a probable

transition of normal goblet cells into undifferentiated non-mucin-containing cells. The increased power of proliferation following the loss of physiological function eventually leads to the formation of a stratified epithelium in glands of the intestinal adenoma.

Wechselmann [15] also concludes that there are two groups of intestinal adenomatous growths, one consisting of a hyperplasia of normal mucosal elements and the other composed of undifferentiated cells. He feels absolutely certain that the second group is due to abnormalities originating independently of catarrhal affections in accordance with Cohnheim's theory. He also gives evidence of a hereditary relation, citing cases where as high as six members of one family showed intestinal adenomatosis. He strongly opposes the etiological conception of susceptibility plus chronic inflammation. Oseki [16] found no evidence of inflammatory alterations in his material and therefore considers intestinal adenomas as neoplastic formations.

Ribbert's [17] views are similar to those of Cohnheim and Wechselmann. He found no indication of inflammation in the neighborhood of intestinal polypi and therefore opposes Verse's views regarding the part played by chronic catarrhal inflammation. Ribbert notes a sharp line of demarcation between the mucin and non-mucin-producing cells and opposes Verse's views supporting the transition of normal epithelium into undifferentiated non-mucin-producing cells found in adenomatous glands. The conception of disturbed development he believes is further supported by the frequent ability to trace polypi back to childhood. Borelius and Sjövall [18] attribute these newformations to an inflammatory process, although they concede the existence of an individual local disposition.

Case Reports

Case 1. A female pig 9 month's old, weighing about 80 pounds, was submitted from a herd in which enteritis due to *Salmonella suipestifer* and coccidia had occurred frequently during the past. The only history available was that the pig had weighed more previously, showed diarrhea, and that it gradually became emaciated. At the time of autopsy the animal was in a poor state of nutrition. The haircoat was rough and dry, and the visible mucous membranes were pale. No evidence of a recent diarrhea was found. A hazelnut-

sized area of pneumonia and edema was found in the apex of each lung. The mucosa of the stomach was rough and thickened, suggesting a previous acute gastritis. The small intestine was devoid of food, anemic and its villi prominent. The wall of the ileum was slightly hypertrophied. The ileocecal valve was completely overgrown by a mass of polypi (Fig. 1). The wall of the cecum was greatly thickened, the mucosa in most places being obliterated by diffuse polypous growths. Many of these tumors were round or irregular, some being over one-half inch in diameter. The large colon presented a similar picture with the exception of the last 18 inches. Beyond this point appeared smaller growths, some suggesting proliferations of the glands in folds of the mucosa. Posterior to this region only scattered esophagostomum nodules were present. The small colon and rectum possessed a yellowish gray mucosa with an absence of gross tumors. The cecal and colic lymph nodes were greatly enlarged. The cut surfaces bulged and presented a yellow and gray mottled appearance. The splenic follicles were enlarged. Other organs showed no significant changes.

Microscopic Examination: The stomach showed a catarrhal gastritis of considerable standing, but an absence of gland proliferation. The small intestine presented a marked interstitial cellular infiltration with intact mucosa and an absence of new gland formations. The polypous growths involving the ileocecal valve, cecum and large colon consisted of adenomatous tissue. The new-formed glands were much larger than normal intestinal glands, the epithelial proliferation producing lateral branches or blind sacs (Figs. 2 and 3). Often the proliferating glands were forked and winding or they formed tree-like structures. Goblet cells were uniformly absent in the new-formed glands constituting the polypous growths. In their place were found undifferentiated, tall, columnar non-mucin-containing cells which were closely packed. Often the nuclei were stratified (Fig. 3).

In a number of preparations examined, single goblet cells were rarely noted in the midst of extensive adenomatous tissue proliferations composed of undifferentiated epithelium. That this proliferating undifferentiated epithelium is capable of occasionally reverting back to the goblet cell type seems to lend support to the conception of the close relationship between the two types of cells, and might be considered as favoring the view that the undifferentiated epithelium

of polypi originates from normal gland epithelium which has undergone degeneration, rather than as originating from misplaced rudimentary cells. In the adjacent uninvolved parts of the intestinal mucosa the glands were lined by goblet cells which were often in a state of hyperactivity.

Cyst formation was common in the polypi. The epithelial cells lining the cysts were shorter and broader, the nuclei being less closely placed than in the tall columnar epithelium. The basement membranes of the new glands were intact and no evidence was found in any of the preparations of this case to suggest pathological malignancy, although the process must be considered as one of clinical malignancy. The pedicles of the polypi were short and consisted of well developed connective tissue structures emanating from the mucosa and submucosa, breaking up the muscularis mucosa and pulling the ends upward. The connective tissue portion of the pedicles divided, sending branches to different parts of the polypi to form a framework (Fig. 2). The mucosa and submucosa were often the seat of inflammatory processes, some of long duration. The adenomatous proliferation did not extend into the deeper structures. The lymph sinuses were distended.

Unlike the two subsequently described cases, which were associated with an active destructive enteritis process, this case showed a more or less definite line of demarcation between the new-formed tissue and the remnants of uninvolved mucosa. In this animal the process did not appear to spread progressively into the uninvolved area by the metaplasia of normal cells, further growth taking place by proliferation of the already degenerated epithelium, sometimes crowding out adjacent normal glands by pressure.

It was from such cases as this that some workers drew the conclusion that there can be no transformation of normal epithelium to this type as a result of destructive or inflammatory processes and that the undifferentiated cells originate from embryonic rudiments. The outer edges of the polypi often were involved by varying degrees of necrosis and covered by an exudate containing *Balantidium coli*. The location of the protozoa noted at this stage of the process did not suggest a correlation with the glandular epithelial proliferation.

The lymph nodes of the cecum and colon showed an advanced diffuse lymphadenitis. The trabecular tissue was separated by an

edematous fluid and infiltrated by leucocytes. The follicles were greatly enlarged and in nearly all instances the germ centers extended close to the periphery and showed endothelioid cell proliferation.

CASE 2. A female pig weighing about 100 pounds and presenting clinical symptoms of infectious enteritis was killed in a moribund condition. Infectious enteritis designates a form of enteritis produced by the *Salmonella suipestifer* as the primary factor and the *Actinomyces necrophorus* as a constant secondary invader.[19] The wall of the ileum was greatly thickened and congested. In most places the mucosa appeared raw and granular with floccules of caseated tissue firmly adherent to the surface. The cecum was slightly involved, while the anterior portion of the large colon was greatly thickened and in places covered by a diphtheric membrane. Incision through these areas revealed a heavy cellular structure which suggested a newgrowth in the mucosa. This condition diminished further back but the caseated membranes were still present. In addition to small areas of pneumonia and the presence of lung worms, the concomitant pathological changes usually found in other organs in infectious enteritis were present in this case.

Microscopic Examination: The tumefied portions of the wall of the ileum and large colon consisted of new-formed, adenomatous tissue. The glands were sometimes forked or showed numerous branches in contrast to the straight simple glands of the normal mucosa. In place of goblet cells the new-formed glands were lined by undifferentiated columnar epithelium similar to that described in the first case. At the edges of the adenomatous tissue there appeared transitions from one type of epithelium to another. In the deeper portions of some glands goblet cells were noted, while above was found a gradual or at times an abrupt change from goblet cells to undifferentiated cells. In the structures situated above the transitional areas the glands were lined by undifferentiated cells. Sometimes the newgrowth crowded out normal glands by pressure. In places, hypertrophic glands lined by goblet cells located above the muscularis mucosa were surrounded by adenomatous tissue. Evidence of an extensive mucosal destruction by a severe enteritis was noted in this case. In places the muscularis mucosa was involved by the retrograde process, although the adenomatous growth did not invade the deeper structures, the glandular proliferation taking

place chiefly in the upper mucosal structures. The surface of the newgrowth showed erosions and cellular infiltration, and was covered by a necrotic exudate which in places contained numerous *Balantidium coli*. Isolated glands contained single specimens of this protozoön.

CASE 3. On the following day another emaciated pig, weighing about 90 pounds, and showing symptoms of severe enteritis was killed in a moribund condition. The diarrheal excreta contained some blood. The usual alterations found in severe infectious enteritis were noted. The changes pertinent to this study were found in the large colon. In addition to areas showing hemorrhagic infiltration and diphtheric membrane some parts of the wall were greatly thickened. Like the previous case the increased thickness of the wall and the elevations of the mucosa consisted of a cellular structure suggesting a newgrowth.

Microscopic Examination: Microscopic study revealed a widespread destruction of the mucosa and extensive granulations often involving all structures above the inner circular muscle. The thickened parts of the large colon consisted of adenomatous proliferations, many of which were situated adjacent to ulcerated areas. The primary destruction involved the deeper structures of the wall as was evidenced by the extensive granulation tissue formation extending to the inner circular muscle. Under the adenomatous proliferations the submucosa was not completely destroyed. It is possible that in the latter areas some glandular tissue remained in the mucosa above from which regeneration and further proliferation could start, while in the ulcerated areas all epithelial or glandular structures were destroyed over a large area, permitting no chance for regeneration from any preëxisting epithelium. On the opposite edge of the ulcer the destructive process extended deeply, but left a portion of the submucosal structure above which glandular proliferation took place. Here the proliferative process had not advanced so far as in the first described field as shown by the smaller amount of newgrowth, small glands and less pronounced branchings. Goblet cells were also absent in this area. Characteristic branchings of glands in the more advanced parts of the adenomatous growths were found. As a rule the interglandular tissue was not so abundant in the newer parts of the proliferations near the lumen. Often the new-formed glands almost approximated, or were separated by only one or two

strands of fibroblasts or leucocytes. This case also showed the transitional pictures in which both goblet and undifferentiated cells appeared in the same gland identical with Case 2.

Experimentally Infected Cases

This acute form of dysentery was reproduced in twelve pigs by feeding intestinal contents and scrapings from the mucosa of the cecum or large colon of naturally infected swine. The tissues of four animals from this experimentally infected group were studied microscopically. The same epithelial proliferative process described in previous cases was found in the cecum and large colon of these four animals.

Epithelial Regeneration in Severe Infectious Enteritis

During the course of our studies on the pathology of infectious enteritis in swine [19, 20] we sometimes observed in cases of extensive mucosal destruction histological pictures where the remaining epithelium of the intestine was given over entirely to the process of regeneration. From islands of altered columnar cells, remnants of a preëxisting normal mucosa, long strand-like cells stretched out over a denuded area in an attempt to cover it. The remnants of injured columnar cells from which the proliferation had its origin possessed large nuclei and an absence of mucin. The elongated proliferating cells reaching out into the denuded necrotic mucosa also had large oval nuclei and contained no mucin. The ability of such regenerated epithelium to repossess functional properties is an important point to consider in connection with diagnosis in unthrifty animals which have previously passed through a severe enteritis. Many such animals recovered and made considerable gain in weight, while others remained stunted. After the acute enteritis process had subsided the mucosa appeared thin and rough in places, readily distinguishable from a normal mucous membrane. An absence of goblet cells was often noted, together with epithelial regeneration. This fact, together with observations on adenomatous proliferations of the human intestine, would suggest that the manner of epithelial regeneration following the subsidence of an infectious or parasitic disease is of importance equal to the inflammatory and destructive

processes during the active stage of the disease. Owing to the short period of time elapsing before swine are killed for food it is possible for advanced adenomatous formations to pass by unnoticed, which may in part explain the rarity of its recorded presence in swine in contrast to the numerous reports of occurrence in the horse, cattle and man.

Summary

1. In experimental and field cases of infectious enteritis in which extensive mucosal destruction occurred, epithelial proliferation originating in the remaining islands of injured cells was noted. These proliferating cells were flattened and elongated with large nuclei. They stretched out into the necrotic tissue in an attempt to cover the denuded mucosa. Swine killed immediately after the subsidence of the infectious enteritis process usually showed an absence of goblet cells in the glands of the large colon, and in their place appeared either low or tall columnar epithelium with large nuclei.

2. An extensive destruction of mucosal tissue in two severe advanced cases of enteritis was associated with a degeneration of the epithelium and the formation of adenomatous growths. A transition from goblet cells to undifferentiatied non-mucin-containing cells was found in some glands at the edges of the adenomatous tissue. In the advanced case showing adenomatous polypi, evidence of a previous inflammatory process was noted. Its etiology could not be determined from the microscopic study, although the herd history would suggest infectious enteritis. The blood serum of this subject agglutinated a *Salmonella suipestifer* antigen in a titre of 1:25. The rare presence of isolated goblet cells situated in large masses of newformed glands lined by undifferentiated, non-mucin-containing cells is further evidence of a close relationship between the normal cells and those of the adenomatous growths. Newgrowths made up of glands lined entirely by goblet cells, described by some workers, were not encountered by us. The tumefied tissue consisted of glands lined by undifferentiated cells.

3. The characteristic bile duct proliferations in coccidiosis of the liver of rabbits, the adenomatous formations in the equine stomach due to nematodes, adenomas in bilharziosis and the characteristic columnar-cell-lined adenomatous proliferations described in connection with lung worm infestations and pneumonia of sheep [21, 22] all

suggest a relationship between intestinal adenomatous proliferations and destructive processes followed by epithelial regeneration, as against their interpretation as independent tumors arising from isolated or misplaced embryonic rudiments.

REFERENCES

1. Kaufmann, E. Lehrbuch der pathologischen Anatomie. (Translated by S. P. Reimann.) P. Blakiston's Son & Co., Philadelphia, 1929, **1**, 774, 775.
2. Petit, G., and Germain, R. Structure et evolution des tumeurs à Spiropteres de l'estomac du cheval. *Bull. Soc. centr. de méd. vét.*, 1907, **61**, 410–417.
3. Petit, G., and Germain, R. Adenomes vermineux de l'estomac du cheval. *Bull. Soc. centr. de méd. vét.*, 1907, **61**, 421–427.
4. Neumann, L. G. A Treatise on the Parasites and Parasitic Diseases of the Domesticated Animals. (Translated by G. Fleming.) W. R. Jenkins Co., New York, 1910, 344.
5. Bergmann, A. M. Körtelpolyper, adenoma tubulare polyporum, i groftarm af svin. *Svensk Veterinärtidskrift*, 1907, **12**, 399–403.
6. Henk, Anton. Wiederholte Darmblutungen bei einem Schweine zufolge Dickdarm adenomen. *Allatorvosi Lapok*, 1927, **50**, 266–267.
7. Port, Konrad. Multiple Polypenbildung im Tractus intestinalis. *Deutsche Ztschr. f. Chir.*, 1895, **42**, 181.
8. Hauser, G. Ueber Polyposis intestinalis adenomatosa und deren Beziehungen zur Krebsentwicklung. *Deutsches Arch. f. klin. Med.*, 1895, **55**, 429.
9. Ruffer, M. A. Notes on the lesions produced by oxyuris vermicularis. *Brit. M. J.*, 1901, **1**, 208.
10. Borst, Max. Die Lehre von den Geschwülsten. J. F. Bergmann, Wiesbaden, 1902, **2**, 541–544.
11. Milton, Frank. Bilharziosis surgically considered. *Lancet*, 1903, **1**, 866.
12. Hart, Carl. Ueber die primäre Multiplizität bösartiger Geschwülste des Verdauungstraktus und ihre Beziehungen zur Polyposis intestinalis adenomatosa. *Ztschr. f. Krebsforsch.*, 1907, **5**, 481–493.
13. Doering, Hans. Die Polyposis intestini und ihre Beziehung zur carcinomatösen Degeneration. *Arch. f. klin. Chir.*, 1907, **83**, 194–227.
14. Verse, Max. Über die Entstehung, den Bau und das Wachstum der Polypen, Adenome und Karzinome des Magen-Darmkanals. Arbeiten aus dem pathologischen Institut zu Leipzig, 1908, **5**, 1–175, S. Hirzel, Leipzig.
15. Wechselmann, Ludwig. Polyp und Carcinom im Magen-Darmkanal. *Beitr. z. klin. Chir.*, 1910, **70**, 855–904.

16. Oseki, S. Polyposis intestini adenomatosa mit Carcinoma recti. *Deutsche Ztschr. f. Chir.*, 1912, **118**, 463.

17. Ribbert, Hugo. Geschwulstlehre für Aerzte und Studierende. Friedrich Cohen, Bonn, Ed. 2, 1914, 503–509.

18. Borelius, J., and Sjövall, E. Ueber Polyposis intestini. *Beitr. z. klin. Chir.*, 1916, **99**, 424–454.

19. Murray, Chas., *et al.* Studies on infectious enteritis in swine. *J. Am. Vet. M. A.*, 1927, **72**, 34–89.

20. Biester, H. E., *et al.* Studies on infectious enteritis in swine: II. The pathogenesis of infectious enteritis. *J. Am. Vet. M. A.*, 1928, **72**, 1003–1022.

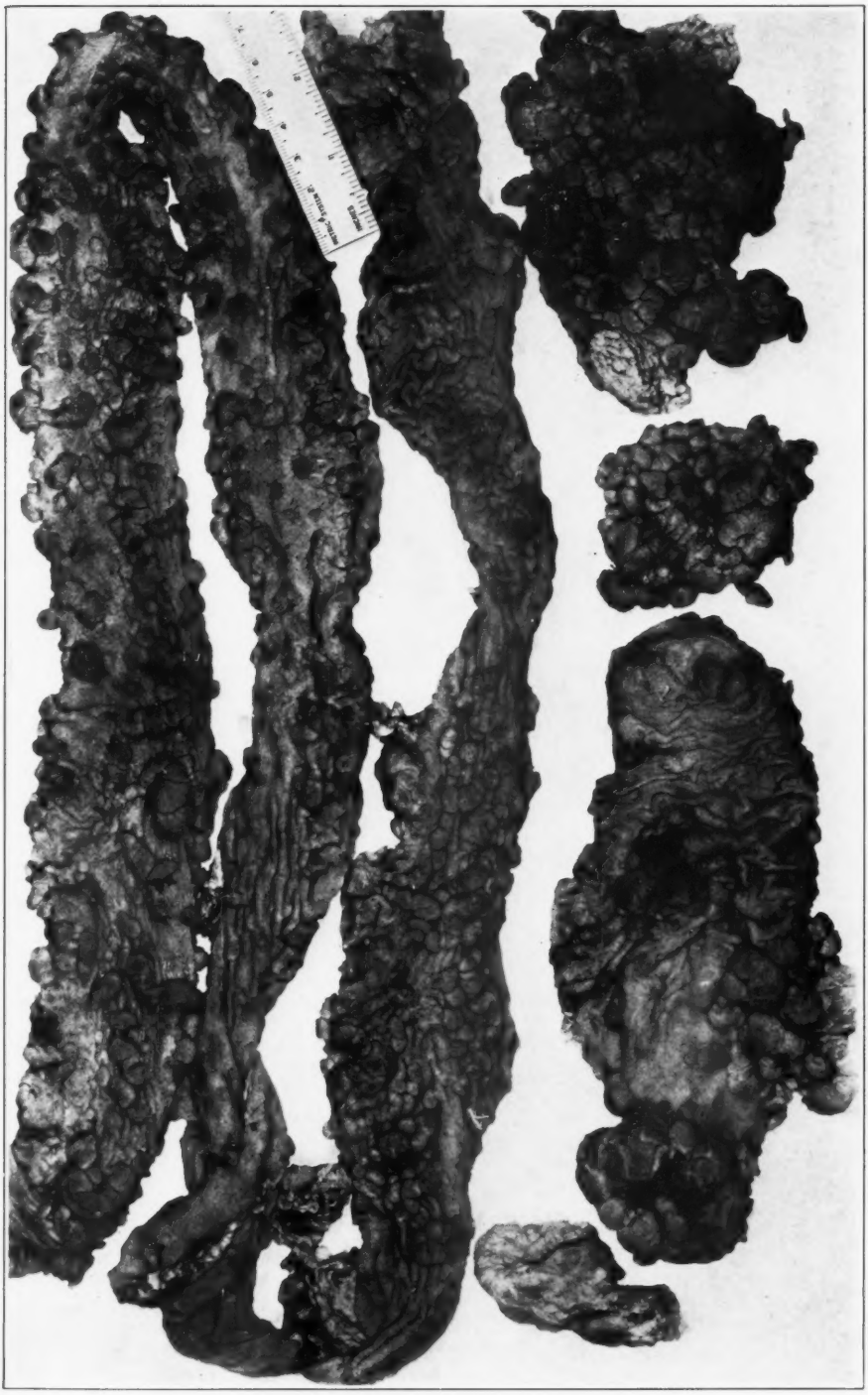
21. M'Fadyean, Sir J. Transformation of alveolar epithelium in verminous pneumonia in the sheep. *J. Comp. Path. & Therap.*, 1920, **33**, 1–10.

22. Pallaske, G. Zur Kenntnis der sogenannten Adenomatose oder der Multiplen Adenome der Lunge des Schafes. *Berl. tierärztl. Wchnschr.*, 1929, **45**, 677–685.

DESCRIPTION OF PLATES

PLATE 35

FIG. 1. Adenomatous polypi in the mucosa of the ileocecal valve, cecum and large colon. Case 1.



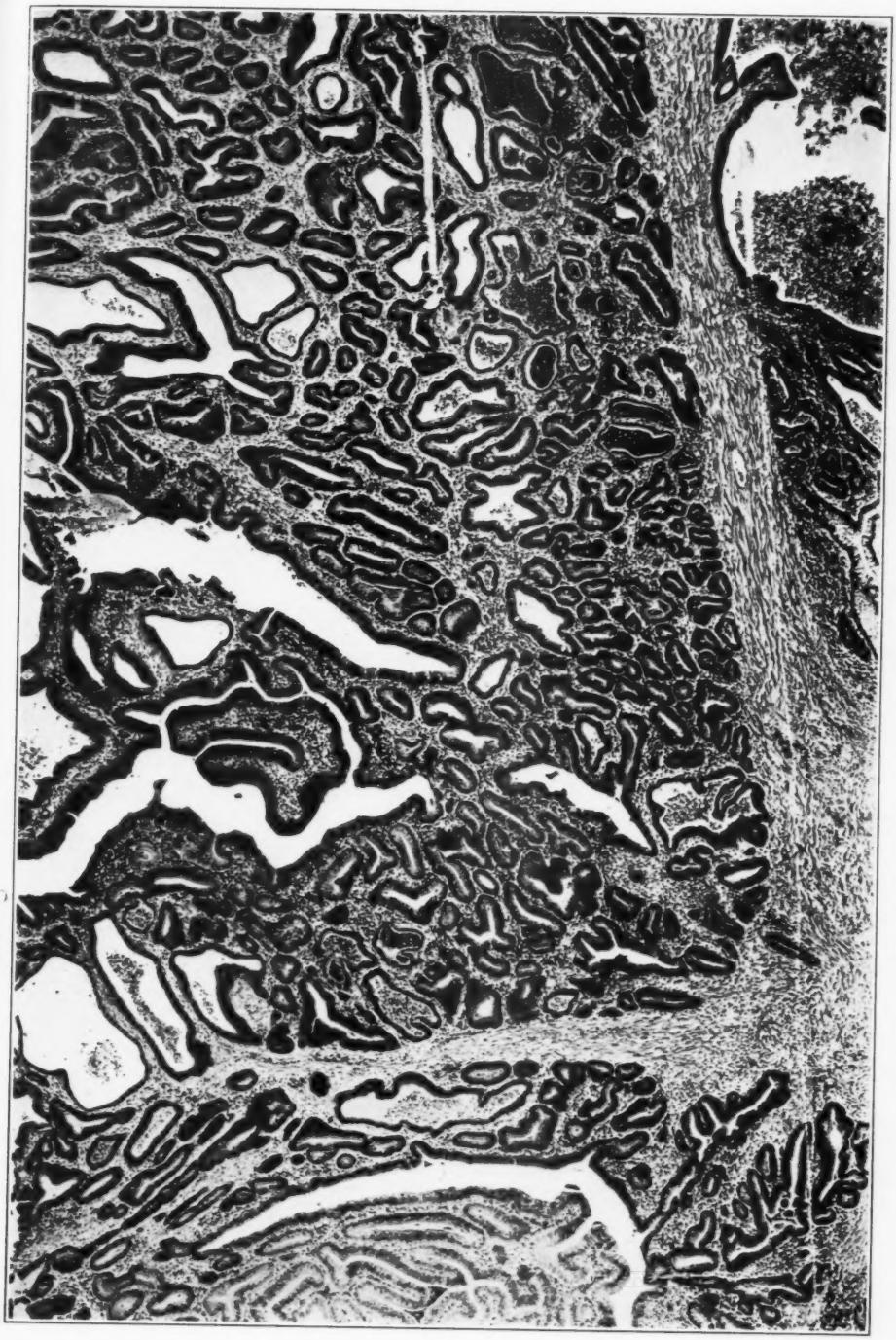
1

Biester and Schwarte Intestinal Adenoma in Swine

PLATE 36

FIG. 2. Branching and cystic glands in the polypous growths from the cecum. Shows the connective tissue framework which emanates from the mucosal and submucosal connective tissue. × 46.

Sand, soil or cinders are commonly ingested by swine. Particles of these become embedded in the exudate or mucosa and cannot be removed by washing. This accounts for the frequent tears found in the sections. It was often necessary to remove hard particles from the block while cutting.

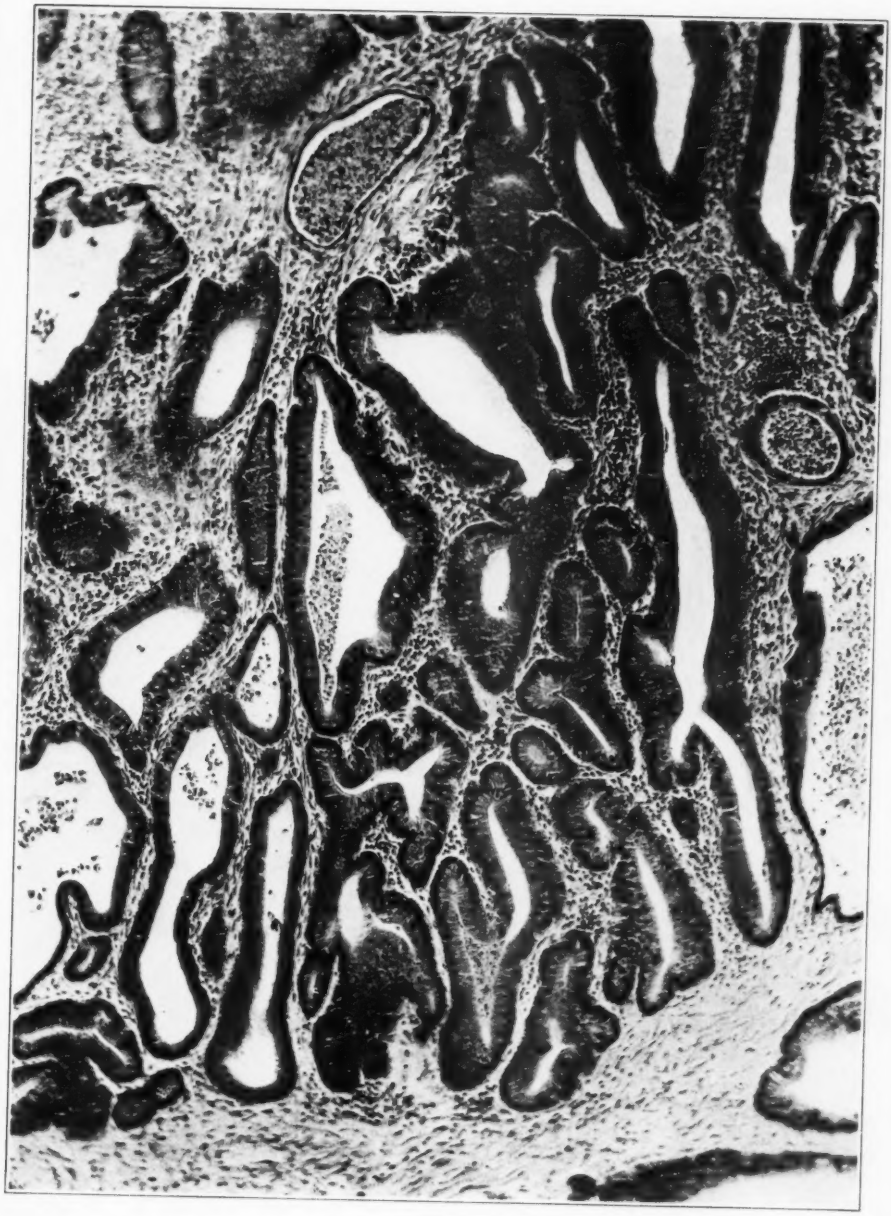


2

Biester and Schwarte Intestinal Adenoma in Swine

PLATE 37

FIG. 3. Large new-formed branching glands lined by undifferentiated columnar epithelium. The nuclei are closely packed and sometimes appear stratified. Goblet cells are absent. × 110.



3

Biester and Schwarte Intestinal Adenoma in Swine